Characterization of a glycosylphosphatidylinositol-bound cell-wall protein (GPI-CWP) in *Yarrowia lipolytica*

Lahcen Jaafar and Jesús Zueco

Unidad de Microbiología, Facultad de Farmacia, Universidad de Valencia, Avda Vicente Andrés Estelles s/n, 46100-Burjassot (Valencia), Spain

The structure and composition of the cell wall of yeast has so far been studied mainly in *Saccharomyces cerevisiae*. It is basically made up of three components: β-glucans, chitin and mannose-containing glycoproteins, also called mannoproteins. Most covalently bound cell-wall mannoproteins belong to the so-called glycosylphosphatidylinositol cell-wall protein (GPI-CWP) family, cell-wall proteins that are bound through the remnant of a GPI residue to 1,6-β-glucan.

The non-conventional yeast *Yarrowia lipolytica* shares Generally Regarded As Safe (GRAS) status with *S. cerevisiae*, has some industrial applications and is increasingly being proposed as a host for the production of recombinant proteins and as a model in the study of dimorphism. However, very little information on cell-wall structure and composition is available for this organism. Here is described the isolation and characterization of *YICWP1*, a homologue of the CWP1 gene from *S. cerevisiae*, which encodes a GPI-CWP, and the identification of its gene product. *YICWP1* encodes a 221 aa protein that contains a putative signal peptide and a putative GPI-attachment site. It shows 28.5% overall identity with Cwp1 of *S. cerevisiae* and a hydropathy profile characteristic of GPI-CWPs. Disruption of *YICWP1*, both in the wild-type and in an mnn9 glycosylation-deficient background, led to the identification of Ylcwp1 as a 60 kDa polypeptide present in cell-wall extracts. To the authors’ knowledge, this is the first report of a GPI-CWP in *Y. lipolytica*, and it suggests that the cell-wall organization of *Y. lipolytica* is similar to that of *S. cerevisiae*.

**INTRODUCTION**

The structure and composition of the cell wall of yeast has so far been studied mainly in *Saccharomyces cerevisiae* and to a lesser extent in *Candida albicans*. It is basically made up of three components: β-glucans, chitin and mannose-containing glycoproteins, also called mannoproteins (Klis, 1994). The mannoproteins themselves can be divided into three groups according to the linkages that bind them to the structure of the cell wall: (i) non-covalently bound, (ii) covalently bound to the structural glucan and (iii) disulfide-bound to other proteins that are covalently bound to the structural glucan of the cell wall (De Nobel & Lipke, 1994; Kapteyn *et al.*, 1999). Covalently bound mannoproteins are normally referred to as cell-wall proteins (CWPs) and fall into two different categories, Pir-CWPs and GPI-CWPs (Kapteyn *et al.*, 1999). Pir-CWPs belong to the PIR (protein with internal repeats) gene family (Toh-e *et al.*, 1993) and are supposed to be bound to 1,3-β-glucan in the cell wall through a kind of linkage sensitive to mild alkali treatment, although some Pir-CWPs have been reported as disulfide-bound CWPs (Moukadiri *et al.*, 1999; Moukadiri & Zueco, 2001). GPI-CWPs receive a glycosylphosphatidylinositol (GPI) anchor during their passage through the secretory pathway and, in addition, they become N-glycosylated and/or O-glycosylated (Orlean, 1997). The addition of the GPI anchor happens at the endoplasmic reticulum and substitutes a hydrophobic domain present at the carboxy terminus of CWPs (Caro *et al.*, 1997). GPI-CWPs, once exported, become attached to 1,6-β-glucan chains through a remnant of the GPI anchor (Lu *et al.*, 1995; Kollár *et al.*, 1997). The 1,6-β-glucan side-chains are linked to 1,3-β-glucan which may be in turn linked to chitin, in a basic structure that repeats itself to give the overall shape of the cell wall (Klis *et al.*, 1997; Orlean, 1997; Lipke & Ovalle, 1998; Kapteyn *et al.*, 1999). In addition, some GPI-CWPs, such as Cwp1, in conditions of low environmental pH, may also bind directly to 1,3-β-glucan through an alkali-sensitive linkage, presumably in a Pir-CWP-like fashion (Kapteyn *et al.*, 2001; Klis *et al.*, 2002). This basic model of structure seems to apply also for *C. albicans* (Kapteyn *et al.*, 2000).

Abbreviations: CWP, cell-wall protein; GPI, glycosylphosphatidylinositol; Pir, protein with internal repeats.

The GenBank accession number for the *YICWP1* gene sequence reported in this article is AY084077.
Yarrowia lipolytica is one of the most extensively studied non-conventional yeasts and constitutes a good alternative model for the study of dimorphism (Barth & Gaillardin, 1997; Hurtado et al., 1999, 2000). However, relatively little is known about its cell-wall structure, especially at the level of CWPs. So far, only two CWPs, Ywp1 and Ylpir1, have been characterized (Ramon et al., 1996; Jaafar et al., 2003a). Ywp1 is reported to be specific to the mycelial cell wall and to be covalently linked to the cell-wall structure, although it does not contain the features characteristic of Pir- or GPI-CWPs, whilst Ylpir1 is the homologue of Pir4 of S. cerevisiae and can be extracted from the cell wall by reducing agents.

In this work, we describe the isolation and characterization of YICWPI, a homologue of the CWP1 gene from S. cerevisiae that encodes a GPI-CWP (Van der Vaart et al., 1995), the isolation and characterization of ylcwp1Δ strains both in a wild-type and in a ylnmn9 background and the identification of the Ylcwp1 polypeptide encoded by YICWPI.

**METHODS**

**Strains and media.** Escherichia coli DH5α was used for the propagation of plasmids; it was grown in Luria broth supplemented with 100 μg ampicillin ml⁻¹ when necessary. E. coli Y1090 was used in experiments involving λgt11. Strain POJA (MATA leu2-270 ura3-202) of Y. lipolytica (C. Gaillardin, Laboratoire de Génétique des Micro-organismes, INRA-CNRS, Thiverval-Grignon, France) was used in all experiments involving Y. lipolytica. Yeast strains were grown in YPD (1 % yeast extract, 2 % Bacto Peptone, 2 % glucose) or synthetic minimal medium (MM; 0.1 % yeast nitrogen base, agar, yeast extract, peptone and yeast nitrogen base Reagents). Screening of λgt11 expression libraries. About 300,000 plaques containing inserts of a mean size of 1 kbp from a Y. lipolytica (yeast morphology) cDNA library in λgt11 (provided by Eulogio Valentin, and obtained by Rosario Gil, Daniel Gozalbo and Eulogio Valentin, Unidad de Microbiología, Facultad De Farmacia, Universidad de Valencia) were screened with a polyclonal antibody that reacts with Pir-CWPs of S. cerevisiae (Moukaddir et al., 1999). The screening of the library was done by the procedures described by Huyhn et al. (1985). The inserts of interest contained in the positive clones were recovered by PCR using the M13 forward and M13 reverse primers and subcloned in pGEMT-easy vectors (Promega).

**Transformation of strains, DNA isolation and sequencing.** Basic DNA manipulation and transformation in E. coli was performed as described by Sambrook et al. (1989). Yeast transformation was carried out by the lithium acetate method (Ito et al., 1983; Gietz & Sugino, 1988). Plasmid DNA from E. coli was prepared using the Flexi-Prep kit (Pharmacia) and DNA fragments were purified from agarose gels using the Sepahgag Band-Prep kit, also from Pharmacia. Sequencing was performed using AmpliTaq polymerase with a Dye Terminator kit (Perkin Elmer) in an Applied Biosystems 373A automatic sequencer.

**Isolation of genomic DNA.** The cells of an overnight 40 ml culture at 28 °C in YPD were harvested, washed in sterile distilled water and incubated for 2 h at 37 °C in 10 ml SEB buffer (0.9 M sorbitol, 0.1 M EDTA, 0.8 % β-mercaptoethanol) containing 5 mg Zymolyase 20T (Seikagaku Kogyo Co.). Protoplast formation was monitored by phase-contrast microscopy. The protoplasts were harvested and resuspended in 3 ml TE buffer (10 mM Tris/HCl pH 7.5, 0.1 mM EDTA); 300 μl of 10 % SDS were added and the samples were incubated for 30 min at 65 °C. Then, 1 ml of 5 M potassium acetate was added and the samples were kept in ice for 1 h. The supernatant was recovered after centrifugation and DNA was precipitated by adding 0.1 vols of 3 M sodium acetate and 2.5 vols ethanol at −20 °C for at least 1 h. The DNA was recovered by centrifugation, resuspended in 3 ml TE, extracted with phenol/chloroform, precipitated again as above and resuspended in 500 μl TE buffer. DNA concentration was determined using a GeneQuantII spectrophotometer (Amersham-Pharmacia).

**Southern analysis.** Samples of genomic DNA (25 μg) were digested with restriction enzymes and the resulting fragments were separated by electrophoresis in 0.8 % agarose gels in TAE buffer (40 mM Tris/HCl pH 7.6, 5 mM sodium acetate, 1 mM EDTA). The agarose gels were then submerged in 0.25 M HCl for 15 min twice, in 0.5 M NaOH, 1.5 M NaCl for 30 min and, finally, in 0.5 M Tris/HCl pH 7, 1.5 M NaCl for a further 30 min. The DNA was then transferred onto a positively charged nylon membrane (Roche or Amersham Biosciences) by capillarity, and the membrane was baked at 120 °C for 30 min to ensure DNA immobilization. Pre-hybridization was performed in 5 × SSC, 0.1 % N-laurylsarcosine, 0.02 % SDS, 1 % Blocking Reagent (Roche Prehybridization Solution) for 1 h at 42 °C. The blot was then hybridized with a digoxigenin (DIG)-labelled DNA probe, which had previously been prepared according to the protocols provided by the manufacturer (Roche), at a concentration of 20 ng ml⁻¹ in Prehybridization Solution for at least 16 h at 42 °C. The membrane was then washed twice in 0.1 % SDS at 68 °C for 15 min at room temperature, and twice more in 0.1 × SSC, 0.1 % SDS at 68 °C. Detection of the hybridized probe was carried out according to the manufacturer’s instructions for the DIG-DNA labelling and detection kit (Roche).

**Phenotypic analysis of the ylcwp1Δ strains.** Calcofluor white and Congo red sensitivities were tested by streaking cells onto plates containing different concentrations of these substances. Samples (2 μl) of serial 1/10 dilutions of cells grown overnight in YPD and adjusted to OD₆₀₀ 8 were deposited onto the surfaces of YPD plates containing different concentrations of Calcofluor white or Congo red, and growth was monitored after 3 days.

**Isolation of cell-wall mannoproteins.** Cell walls from Y. lipolytica were purified and extracted with Zymolyase 20T as follows. Cells in the early-exponential phase were harvested and washed twice in 10 mM Tris/HCl pH 7.4, 1 mM PMSF (buffer A). The harvested biomass was resuspended in buffer A in a proportion of 2 ml (g wet weight)⁻¹; glass beads (0.45 mm in diameter) were added up to 50 % of the final volume, and the cells were broken by shaking four times for 30 s, with 1 min intervals, in a CO₂ refrigerated MSK homogenizer (Braun Melsungen). Breakage was confirmed by phase-contrast microscopy and the walls were washed six to eight times in buffer A. Removal of non-covalently bound proteins was achieved by boiling the walls in buffer A containing 2 % SDS [10 ml (g walls, wet weight)⁻¹] for 10 min, followed by six to eight washes in buffer A. The purified cell walls were then extracted in buffer A containing 500 μg Zymolyase 20T ml⁻¹, using 10 ml (g walls, wet weight)⁻¹, for 3 h at 30 °C in an orbital incubator at 150.
200 r.p.m. The extract was separated from the cell walls by centrifugation and concentrated 20-fold using a Centriprep-10 concentration device (Amicon/Millipore).

**SDS-polyacrylamide gels and Western-blot analysis.** Proteins were separated by SDS-PAGE according to the method of Laemmli (1970) in 10 or 12 % polyacrylamide gels. The proteins separated by SDS-PAGE were either stained with Coomassie brilliant blue or transferred onto Hybond-C nitrocellulose membranes as described by Towbin et al. (1979) and Burnette (1981). Membranes were blocked overnight in Tris-buffered saline containing 0-05 % Tween 20 (TBST) and 5 % non-fat milk. The blocked membranes were washed three times in TBST and incubated for 1 h in TBST containing the antibody at a dilution of 1 : 5000. After three washes in TBST, the membranes were incubated for 20 min in TBST containing goat anti-rabbit IgG-peroxidase at a dilution of 1 : 12 000 and washed in TBST. Finally, antibody binding was visualized on X-ray film using the ECL method (Amersham).

**RESULTS**

**Isolation of the YICWP1 gene**

In an attempt to isolate Pir-CWPs of *Y. lipolytica*, we proceeded to screen a *sgt11*-based gene library from *Y. lipolytica* using an antibody that reacts with Pir-CWPs from *S. cerevisiae* (Moukadiri et al., 1999). After the screening of some 300 000 plaques (mean insert 1 kbp), we obtained 13 positives, two of which were confirmed after secondary and tertiary screening. These were named L1 and L2, and the inserts contained in them were amplified by PCR, using the M13 forward and reverse universal primers, and sequenced. The two inserts, of 700 and 500 bp, respectively, were found to correspond to the same gene, and comparison of the amino acid sequence encoded by the ORF contained in the longer of the two inserts revealed homology with Cwp1 of *S. cerevisiae*, a GPI-CWP first reported by Van der Vaart et al. (1995). To characterize the chromosome region containing the insert contained in L1, Southern-blot analysis was performed using genomic DNA from *Y. lipolytica* PO1A and the 700 bp L1 insert as probe. The results from this analysis suggested the existence of a single copy of the gene and, at the same time, gave us an idea of the approximate restriction map of the chromosome region containing it. To isolate the complete gene, we prepared a mini-gene library by digestion of genomic DNA from *Y. lipolytica* PO1A with the restriction enzymes *PstI* and *HindIII*. The restriction fragments generated were separated by electrophoresis in a 0-7 % agarose gel; a slice of the gel supposed to contain the DNA fragments of around 3-3 kbp in size was cut out, the DNA fragments were eluted from the gel and ligated in pBlueScript II (Stratagene) vector, previously digested with *PstI* and *HindIII*, to give the mini-gene library. Screening of the mini-gene library, using the L1 insert as a probe, gave a positive clone with a 3-3 kbp insert that was confirmed by PCR to contain the L1 insert. The sequence of the complete gene was obtained using the oligonucleotides LNN1 (5’-TTGGCCCTTAAAACTGCAATG-3’) and LNN2 (5’-TTGGATGTTGAACTTTGCGCCG-3’) which hybridize at the extremes of the known sequence of the L1 insert and which prime outwards from it, and further confirmed using the oligonucleotides LF (5’-TTAACCCAGACTACGAC-3’) and LR (5’-TCCAGTCTCGTGATGGTG-3’) to give a double-strand reading in all the length of the 1456 bp fragment sequenced. Analysis of the sequence revealed the existence of an ORF of 666 bp in length that encoded a putative protein 221 aa long with homology to Cwp1 of *S. cerevisiae* (Van der Vaart et al., 1995). Accordingly, we named this gene *Ylcwp1*.

**Structural analysis of the amino acid sequence encoded by YICWP1**

Alignment of the amino acid sequence encoded by YICWP1 with that of Cwp1p of *S. cerevisiae* (Fig. 1) shows 28-5 % overall identity and the presence of several common features. Ylcwp1 has a putative signal peptide with a possible peptidase site between positions 16 and 17, and a putative GPI-attachment site at the asparagine in position 200 that closely resembles the consensus GPI-attachment signal in *S. cerevisiae* (Nuoffer et al., 1993; Van der Vaart et al., 1995), defined by an asparagine followed by glycine and alanine (NAG or NGA) – NGA in Ylcwp1 – followed by a hydrophobic carboxy-terminal region. In this context, it is important to note that the Kyte and Doolittle hydropathy profiles of Cwp1 and Ylcwp1 are both characteristic of GPI-CWPs (Fig. 2). Other common features are the high content of serine and alanine, 77 out of 239 aa in Cwp1 and 72 out of 221 aa in Ylcwp1, and the presence of the motif DGQIQA close to the carboxy terminus. This feature is shared by at least three GPI-CWPs in *S. cerevisiae*, Cwp1, Cwp2 and Srp1 (Van der Vaart et al., 1995), and is also present in all four Pir-CWPs of *S. cerevisiae* (Toh-e et al., 1993; Moukadiri et al., 1999), being part of the ‘internal repeats’ that give them their name, but not in the single Pir-CWP characterized so far in *Y. lipolytica* (Jaafar et al., 2003a). The presence of the DGQIQA feature would also explain why we have isolated YICWP1, a GPI-CWP, using an antibody that reacts with Pir-CWPs of *S. cerevisiae*.

**Deletion of the YICWP1 gene and characterization of ylcwp1Δ strains**

Disruption ylcwp1Δ strains were created both in wild-type strain PO1A and in mnn9Δ (Jaafar et al., 2003b) backgrounds to determine the possible phenotypes associated with the disruption of the gene. The disruption was performed in both strains using the two-step ‘pop in/pop out’ method (Rothstein, 1991) with *URA3* as marker. For this, the gene and part of its regulatory sequences were amplified with oligonucleotides LF and LR as a 1456 bp DNA fragment that was subcloned in the pGEMT-easy vector (Promega). This construction was then digested with *NarI* and ligated, with the loss of 435 bp of the coding sequence of the gene, and a SalI–SalI fragment containing the *URA3* marker was subcloned in the single SalI site in the construction. Finally, the construction was rendered linear by digestion with *HpaI* to produce the disruption cassette that
was then transformed in the PO1A and mnn9 strains. After monitoring the correct integration of the disruption cassette, a selected clone for each strain was plated onto plates containing 5'-fluoroorotic acid (5'-FOA), and clones derived from these plates and grown on YPD were monitored for the loss of the wild-type allele both by PCR and by Southern analysis. PCR was performed on DNA from colonies growing on YPD plates after the 5'-FOA

**Fig. 1.** Sequence alignment of the predicted amino acid sequence of Cwp1 of *Y. lipolytica* (YICwp1) with that of Cwp1 from *S. cerevisiae*. Percentage identity of YICwp1 with Cwp1 is 28.51%.

**Fig. 2.** Kyte and Doolittle hydropathy profile of Cwp1 (a) and YICwp1 (b).
Fig. 3. Validation of the ylcwp1Δ deletion strains. (a) PCR using the LF2 and LR oligonucleotides to generate either a 1.34 kbp band (parental) or a 905 bp band (disrupted allele). Lanes: M, λ DNA digested with EcoRI and HindIII; 1, ylcwp1Δ strain; 2, ylmmn9Δ ylcwp1Δ strain; 3, strain PO1A. (b) Southern analysis of the parental PO1A (lane 1), ylcwp1Δ (lane 2) and ylmmn9Δ ylcwp1Δ (lane 3) strains. Genomic DNA was digested with restriction enzymes EcoRV and HindIII, separated by agarose electrophoresis, transferred onto nylon membranes and hybridized with the 700 bp PCR-generated L1 insert. M corresponds to λ DNA digested with EcoRI and HindIII. Expected size was 3.5 kbp (parental) or 3.1 kbp (disrupted allele).

Identification of Ylcwp1 in Zymolyase 20T extracts from the cell walls of the mnn9 strain of Y. lipolytica

Finally, based on the described localization of Cwp1 in extracts obtained from cell walls of S. cerevisiae (Van der Vaart et al., 1995; Kapteyn et al., 2000), we proceeded to identify Ylcwp1 in similar extracts from cell walls of the mnn9 strain of Y. lipolytica. This strain was chosen because of the lower degree of glycosylation of its glycoproteins, which makes the identification of individual bands in electrophoresis easier. With this aim, we isolated cell walls from ylmmn9Δ and ylmmn9Δ ylcwp1Δ strains from Y. lipolytica, and treated them sequentially with SDS, to eliminate non-covalently bound proteins, and with Zymolyase 20T, to release GPI-CWPs. The analysis of the electrophoretic pattern of the bands of the Zymolyase 20T extract was performed by Western immunoblot, using antibodies raised against purified cell walls of Y. lipolytica. The comparison of the pattern of bands of the extracts from the cell walls of the ylmmn9Δ and ylmmn9Δ ylcwp1Δ strains shows a single and relatively clear difference that consists of the presence of a band of some 60 kDa in the extract of the ylmmn9Δ strain, a band that is not present in the extract from the double ylmmn9Δ ylcwp1Δ disruptant strain (Fig. 5). Accordingly, we assumed this band to correspond to Ylcwp1.

DISCUSSION

In this work, we present the isolation and characterization of the YLCWPI gene of Y. lipolytica, presumably encoding a GPI-CWP, and the study of the phenotypic effects of the disruption of this gene, both in the parental strain PO1A and in a ylmmn9Δ strain. This gene encodes a 221 aa protein that is homologous to Cwp1, a GPI-CWP of S. cerevisiae (Van der Vaart et al., 1995). Ylcwp1, similarly to Cwp1, contains a putative signal peptide and a putative GPI-attachment signal, consisting of an NGA sequence, compatible with the NGA or NAG sequences found in S. cerevisiae GPI-CWPs (Nuoffer et al., 1993; Van der Vaart et al., 1995), followed by a stretch of hydrophobic amino acids.
that constitutes the amino terminus of the protein, giving an overall hydrophobic profile characteristic of a GPI-CWP. Other common features are the presence of a DGQIQA motif in both the sequence of Cwp1 and Ylcwp1, a motif that is also shared by Srp1 and Cwp2, other GPI-CWPs of *S. cerevisiae* (Van der Vaart *et al.*, 1995), and the high content of serine and alanine in both Cwp1 and Ylcwp1. Taken together, these data strongly suggest that *YlCWP1* encodes a GPI-CWP of *Y. lipolytica*, and clearly point to the existence of a family of GPI-CWPs in *Y. lipolytica* that shares the main features of the GPI-CWPs of *S. cerevisiae*.

However, the initial aim of our work was to isolate additional members of the Pir-CWP family of *Y. lipolytica*, following the characterization of Ylp1, the first Pir-CWP characterized in *Y. lipolytica* (Jaafar *et al.*, 2003a). For this, we screened *gt11*-based *Y. lipolytica* expression libraries using a polyclonal antibody that reacts with Pir-CWPs of *S. cerevisiae* (Van der Vaart *et al.*, 1995), and the high content of serine and alanine in both Cwp1 and Ylcwp1. Taken together, these data strongly suggest that *YICWP1* encodes a GPI-CWP of *Y. lipolytica*, and clearly point to the existence of a family of GPI-CWPs in *Y. lipolytica* that shares the main features of the GPI-CWPs of *S. cerevisiae*.

Disruption of *YICWP1* was performed in two different strains, strain PO1A and a *ylmnn9* strain (Jaafar *et al.*, 2003b). The use of strains harbouring the *mnn9* allele plus the disruption of a specific GPI-CWP-encoding gene has been used recently to highlight co-operative functions in the biogenesis and maintenance of the cell wall in *S. cerevisiae* (Horie & Isono, 2001). Changes in the cell wall of the disruptant *ylcwp1Δ* and of the double disruptant *ylcwp1Δ ylmnn9Δ* were detected by testing their sensitivities to Calcofluor white and Congo red, substances that disturb the cell wall, aggravating the consequences of cell-wall defects (Elorza *et al.*, 1983; Kopecka & Gabriel, 1992; Ram *et al.*, 1994). However, the results of this assay showed a slight increase in the sensitivity to Congo red only, both in the *ylcwp1Δ* and in the *ylcwp1Δ ylmnn9Δ* strains, compared to their respective parental strains. This result is in agreement with that reported for the *cwp1* strain in *S. cerevisiae* where only a slight increase in sensitivity to Calcofluor white and Congo red was detected (Van der Vaart *et al.*, 1995), and confirms the possible role of Ylcwp1 in the cell wall of *Y. lipolytica*. Moreover, we have presumably identified Ylcwp1 as a 60 kDa band present in the Zymolyase 20T extract from cell walls of the *ylmnn9Δ* strain, by comparison with an identical extract obtained from the cell walls of the *ylcwp1Δ ylmnn9Δ* strain. The identification of this 60 kDa

![Fig. 4. Effect of *YICWP1* deletion on the sensitivity to Congo red of the parental (PO1A) and *ylmnn9Δ* strains. Cells of the parental and *ylcwp1Δ* strains (c and d) or the *ylmnn9Δ* and *ylmnn9Δ ylcwp1Δ* strains (a and b) were grown in YPD and 1/10 dilution series of 2 μl droplets of each strain was inoculated onto the surface of YPD plates containing 1 μg Congo red ml⁻¹ (b) or 35 μg Congo red ml⁻¹ (d). (a) and (c) correspond to YPD control plates.](image-url)
The cell-wall extracts were submitted to SDS-PAGE in 10 % acrylamide gels, transferred onto nitrocellulose membranes and incubated with a polyclonal antibody raised against purified cell walls of the yeast form of Y. lipolytica. The arrow points to the band presumably corresponding to Ylcwp1.

Finally, Kapteyn et al. (2001) have reported that, in S. cerevisiae, at low environmental pH, Cwp1 becomes anchored through an alkali-labile linkage to 1,3-β-glucan, instead of, or in addition to, the GPI-derived linkage. In the case of the Ylcwp1 band we detected in the Zymolyase 20T extracts, although we presume it may correspond to GPI-anchored Ylcwp1, we cannot discard the possibility that it represents the protein directly bound to 1,3-β-glucan through an alkali-labile linkage, as described by Kapteyn et al. (2001) for Cwp1 in S. cerevisiae.

ACKNOWLEDGEMENTS

We thank Eulogio Valentin, Daniel Gozalbo and Rosario Gil (Unidad Departamental de Microbiología, Facultad de Farmacia, Universidad de Valencia) for the gift of the Agt11 cDNA library, and Maria Iraino (Unidad Departamental de Microbiología, Facultad de Farmacia, Universidad de Valencia) for the gift of the antibody raised against the purified cell walls of Y. lipolytica. This work was supported by grant BMC2001-2761 from the Ministerio de Ciencia y Tecnología (Spain).

REFERENCES


Huyhn, T. V., Young, R. A. & Davis, R. W. (1985). Constructing and screening of cDNA libraries in S. cerevisiae (Van der Vaart et al., 1995). As is the case with Cwp1, there are no putative N-glycosylation sites in Ylcwp1; however, the discrepancy between the expected molecular mass, as deduced from the amino acid sequence, and that observed in SDS-PAGE could be accounted for by O-glycosylation.


