FTIR spectroscopic analysis of *Saccharomyces cerevisiae* cell walls: study of an anomalous strain exhibiting a pink-colored cell phenotype

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Abstract

A new strain, exhibiting an intriguing pink-colored cell phenotype, was obtained after an encoding α-glucosidase gene from an archaebacteria *Thermococcus hydrothermalis* was cloned by functional complementation of a mal11 *Saccharomyces cerevisiae* mutant TCY70. The possible implications of the α-glucosidase on the cell wall were evaluated by infrared spectroscopy and data indicate a 30% decrease in mannoproteins and an increase in β-glucans. The loss of mannoproteins was confirmed by experiments on cells deprived of peptidomannans. Modifications in the major components of the cell wall did not jeopardize cell viability. Such rapid optical spectroscopic method can be used to screen a wide range of yeast mutants. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

The cell wall of *Saccharomyces cerevisiae* makes up 15–30% of the dry weight of the cell [1] and 25–50% of the volume. The chemical composition is known. It is mainly composed of mannoproteins and β-glucans (85–90% of the cell wall dry mass) and a smaller amount of chitin (1–3%) and lipids (2–5%) [2,3]. The β(1 → 3) glucan–chitin complex is the major constituent of the inner wall. β(1 → 6) glucan links the components of the inner and outer walls [4]. Mannoproteins, which are extensively O and N glycosylated polypeptides, are on the outer surface of the wall and limit wall permeability to solute [5]. The structures of mannans have been described by Peat et al. [6] and Fleet [7]. Chitin contributes to the insolubility of the fibers [8]. The proportion of these components may vary between exponentially growing and stationary phase cells, between parental and mutated lines, during the cell cycle or in response to environmental conditions such as nutrient and oxygen availability, temperature and pH.

Differences in cell wall structure and composition are studied by acid hydrolysis [9] or potassium hydroxide extraction [10], spheroplast rate assay [11] or microscopy [12]. During this decade, physical methods such as Fourier transform infrared (FTIR) spectroscopy have been extensively used to identify and classify microorganisms [13,14]. FTIR data from bacteria have also been correlated with modifications in secondary structures and this in relation with a resistance phenotype [15,16]. Studies concerning yeasts have been reported, but only for classification purposes with a correct identification of 97.5% [17] or for the analysis of homopolymers extracted from cell walls [18].

To our knowledge, there is no FTIR report on *S. cerevisiae* cell wall, although recently a spectroscopic study, in conjunction with statistical methods, has been carried out on 1000 mutagenized flax plants to monitor structural and architectural cell wall alterations [19]. All these investigations are possible because of the ‘fingerprinting’ capabilities of the technique, its non-destructive nature, rapidity, requirement of very little sample, and the molecular level information that is accessible. Furthermore, the hyphenation of FTIR spectroscopy with mathematical and statistical methods has rendered the technique more versatile.

An encoding α-glucosidase gene from an archaebacte-
rium *Thermococcus hydrothermalis* was cloned by functional complementation of a *mal11* *S. cerevisiae* mutant strain TCY70. We reported the isolation, cloning and sequencing in a recent study [20]. The obtained strain (AGLSC) exhibited an intriguing characteristic with pink-colored cells. Structural modifications in the cell wall of the mutated strain AGLSC have been probed by FTIR spectroscopy.

2. Materials and methods

2.1. Yeast strains and yeast growth

*S. cerevisiae* TCY70 (*MAT a, mal11::LEU2, ura3*) yeast strain [21] was generously provided by Dr. Paul Sollitti. The mutated strain AGLSC was obtained by transformation of the TCY70 strain using p41F2 plasmid. p41F2 is the pYME1 [22] vector which contains a 2 kbp archaeabacterial DNA fragment from *T. hydrothermalis*. The α-glucosidase encoding gene is the only ORF in this DNA fragment [20]. In this study TCY70 and AGLSC are respectively considered as the wild-type and mutant strains. In order to consider the impact of the pYME1 vector in the phenotype, we also used the TCY70 strain transformed by pYME1 (TCY(pYME)). Yeast strains were cultured on minimal media (0.67% yeast nitrogen base, 2% glucose) supplemented or not with uracil at 30°C.

2.2. Assimilation and fermentation of carbon sources

This was performed using the API 20E kit (Pasteur Mérieux, France).

2.3. RFLP (restriction fragment length polymorphism) analysis

The fragment of the MET2 gene was amplified by PCR prior to RFLP analysis as described previously [23,24].

2.4. Sterilization of yeast cells

Sterilization was performed in 20 mM citrate buffer in order to solubilize peptidomannans. The method has been previously described by Peat et al. [6].

2.5. FTIR spectroscopy – sample preparation, recording and treatment of spectra

Spectra were recorded on 72 h old yeast colonies with an ATR (attenuated total reflection) kit using a Bomem MB-100 (Vannier, QC, Canada) FTIR spectrometer as described previously [15]. All measurements were acquired (Lab Calc, Galactic Ind. Corp.) in triplicate on two independent cultures, normalized in the 650–1800 cm⁻¹ region and reproducibility was in the range ± 2%. To gain more insight into cell wall structural components we used second derivation and curve-fitting procedures [25]. All spectral and data treatments were performed using LabSpec software (DILOR, Lille, France).

3. Results

3.1. Morphological and physiological identification

Cells from TCY70 and AGLSC strains were observed by optical microscopy (data not shown). No morphological differences were noticed between the two strains. Assimilation and fermentation profiles were performed for the TCY70 and AGLSC strains. The two strains were able to utilize glucose and sucrose but not lactose and cellobiose. Maltose was assimilated by the AGLSC strain only.

3.2. RFLP analysis

In addition to morphological and physiological identification, characterization of yeast species was realized by a PCR-RFLP analysis using a fragment of the MET2 gene. Two primers were used to amplify this region. PCR products show the same length of about 500 bp for TCY70 and AGLSC strains. These PCR products were digested with two restriction endonucleases: *Eco*RI and *Pst*I. Identical restriction digests by the two enzymes were obtained (data not shown). The DNA fragment was cleaved once by *Eco*RI with cleavage products of about 200 and 350 bp and no cleavage by *Pst*I. This restriction profile is described as the profile obtained for a *S. cerevisiae* strain [23,24].

3.3. FTIR spectroscopic analysis of intact cells

Fig. 1 depicts the normalized ATR-FTIR spectra in the frequency range 670–1800 cm⁻¹ of the wild-type strain TCY70 (WT), the WT strain transformed with pYME1 plasmid (TCY(pYME) strain), and the mutated AGLSC strain. The advantage of the ATR mode is that the IR beam penetrates only about 3 μm into the cell, thus giving a good indication of its wall composition. Each spectrum presented is an average of three independent measurements. From this comparison it can be clearly seen that the spectra of the WT and TCY(pYME) strains are very similar and are almost superimposed, while the mutated AGLSC strain exhibits some important spectral modifications. These modifications include: (i) a higher IR absorption intensity in the protein amide I band (mainly CN stretching and contribution of N–H bending) centered at 1639 cm⁻¹, without change in the spectral profile; (ii) decrease in intensity and an upshift of 12 cm⁻¹ in the frequency (new types of bonds or rearrangement of exist...
Fig. 1. Normalized ATR-FTIR average spectra of (dash dot dot dash line) TCY70, (gray solid line) TCY(pYME) and (dotted line) AGLSC strains in the 670–1800 cm⁻¹ range. For each strain, spectra were recorded in triplicate on two independent cultures and for each spectrum 100 scans were co-added at a resolution of 4 cm⁻¹.

### Table 1

Areas and assignments of bands resulting from curve-fitting of the FTIR spectra of TCY70 and AGLSC *S. cerevisiae* strains in the spectral range 790–1190 cm⁻¹

<table>
<thead>
<tr>
<th>Wavenumber (cm⁻¹)</th>
<th>Band areaa (%)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>806</td>
<td>42.15</td>
<td>mannans</td>
</tr>
<tr>
<td>822</td>
<td>7.48</td>
<td>absent</td>
</tr>
<tr>
<td>851</td>
<td>4.26</td>
<td>K-glucans</td>
</tr>
<tr>
<td>860</td>
<td>absent</td>
<td></td>
</tr>
<tr>
<td>879</td>
<td>17.69</td>
<td>mannans</td>
</tr>
<tr>
<td>894</td>
<td>9.27</td>
<td>β-glucans</td>
</tr>
<tr>
<td>911</td>
<td>20.56</td>
<td>mannans</td>
</tr>
<tr>
<td>971</td>
<td>3.58</td>
<td>mannans</td>
</tr>
<tr>
<td>994</td>
<td>14.62</td>
<td>β(1→6) glucans</td>
</tr>
<tr>
<td>1026</td>
<td>23.02</td>
<td>β(1→4) glucans</td>
</tr>
<tr>
<td>1052</td>
<td>19.93</td>
<td>mannans</td>
</tr>
<tr>
<td>1077</td>
<td>19.97</td>
<td>β(1→3) glucans</td>
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<tr>
<td>1103</td>
<td>11.14</td>
<td>β(1→3) glucans</td>
</tr>
<tr>
<td>1141</td>
<td>8.14</td>
<td>β(1→3) glucans</td>
</tr>
</tbody>
</table>

aSpectral regions are surface-normalized to 100% for calculation of band areas.
be used to extract the hidden structural information. Fourier deconvolution, second derivative and curve-fitting are some of the currently used methods [25].

In order to correlate the observed spectral differences with the structural differences that might exist between the TCY(pYME) and AGLSC strains, we have in this work used a curve-fitting procedure on two distinct spectral regions: (i) 790–925 cm$^{-1}$, the ‘fingerprint region’ which informs on the mannans and global glucan content; and (ii) 925–1190 cm$^{-1}$, the polysaccharide absorbing region, which also reveals component structures of mannans and β-glucans. The curve-fitting is based on a least-square method using Gaussian and Lorentzian bands [25]. This procedure calculates a theoretical spectrum which best fits

Fig. 2. Curve-fitting of ATR-FTIR spectra in the frequency range 790–925 cm$^{-1}$; (a) TCY(pYME), $\chi^2 = 2.59 \times 10^{-4}$, and (b) AGLSC, $\chi^2 = 2.45 \times 10^{-2}$. (thick line) and (dotted line) contours represent respectively the experimental and calculated spectra. This spectral region is surface-normalized to 100% for calculation of band areas.
Fig. 3. Curve-fitting of ATR-FTIR spectra in the frequency range 925–1190 cm$^{-1}$: (a) TCY(pYME), $\chi^2 = 1.82 \times 10^{-4}$, and (b) AGLSC, $\chi^2 = 1.9 \times 10^{-2}$. (thick line) and (dotted line) contours represent respectively the experimental and calculated spectra. This spectral region is surface-normalized to 100% for calculation of band areas.
the experimental one. The accuracy of the fit is given by the chi-square value and the lower the chi-square value, the better is the fit.

As can be seen from the spectral decomposition obtained in Fig. 2 and Table 1 (where band areas are calculated), for the first region 790–925 cm\(^{-1}\), there is a dramatic spectral profile change between the control experiment (Fig. 2a) and the mutant strain (Fig. 2b). These changes concern a very strong intensity decrease of the band at 806 cm\(^{-1}\) and to a lesser extent that at 911 cm\(^{-1}\), both corresponding to mannans [18]. At 879 cm\(^{-1}\) the band is shifted to 873 cm\(^{-1}\), with also a strong increase in intensity. Another \(\beta\)-glucan feature near 890 cm\(^{-1}\), present in almost all different types of \(\beta\)-glucan structures, is also found to increase. An intriguing feature is the appearance of an unassigned band at 860 cm\(^{-1}\) in the AGLSC strain. The glycogen content remains unchanged in both strains as indicated by the absorption band at \(\sim 850\) cm\(^{-1}\).

The polysaccharide region is presented in Fig. 3 in the spectral range 925–1190 cm\(^{-1}\). The spectral profile in this region mainly reflects the absorption of sugars present in the yeast cell wall. Indeed, the majority of polysaccharides of the yeast cell is found in its wall [12]. It can be noticed that the two strains exhibit different spectral profiles and the underlying differences are well revealed by a curve-fitting of the spectrum of the control cells (Fig. 3a) and that of the AGLSC ones (Fig. 3b). The corresponding band areas and assignments are also indicated in Table 1. The component bands that are disclosed concern mainly \(\beta\)-glucans at 994 cm\(^{-1}\) (\(\beta(1\rightarrow6)\)), 1026 cm\(^{-1}\) (\(\beta(1\rightarrow4)\)), and 1077, 1103, 1141 cm\(^{-1}\) (\(\beta(1\rightarrow3)\)); and mannans at 971 and 1052 cm\(^{-1}\). This spectral region shows a similar tendency as before for mannann and glucan contents. Bands due to mannans seem to decrease while almost all those due to glucans (\(\beta(1\rightarrow3), \beta(1\rightarrow4), \beta(1\rightarrow6)\)) exhibit an increase in band areas for the mutant strain, except for one \(\beta(1\rightarrow3)\) component at 1147 cm\(^{-1}\) which decreases and is also upshifted by 6 cm\(^{-1}\). The frequency positions of most of the other bands do not vary much except for the mannann component at 971 cm\(^{-1}\) which is downshifted by 6 cm\(^{-1}\) to 965 cm\(^{-1}\).

3.5. FTIR spectroscopic analysis of sterilized cells

In order to validate our spectroscopic observations concerning the cell wall modifications in the AGLSC strain, we have conducted FTIR measurements on TCY(pYME) and AGLSC cells void of peptidomannans after sterilization. This procedure has been employed because the removal of peptidomannans does not affect their characteristic morphological forms [6], which are preserved by the presence of glucans. FTIR spectra of the non-sterilized and sterilized TCY(pYME) and AGLSC strains have been recorded and the average spectrum computed as before. A cursory examination of the spectra shows some appreciable differences in the four strains (data not shown). In order to better appraise the cell wall modifications after sterilization, we display in Fig. 4 the second derivative spectra, which better enhance the spectral differences. This approach allows to confirm some of the observations already made using the curve-fitting procedure.
By comparing Fig. 4a and b, representing the non-sterilized TCY(pYME) and AGLSC strains respectively, the mannan peaks at 912 and 971 cm\(^{-1}\) were found to exhibit a lower intensity in the case of the mutant strain. In the mutant strain, the bands at 991, 1031 and 1079 cm\(^{-1}\), assigned respectively to \(\beta(1\rightarrow6)\), \(\beta(1\rightarrow4)\) and \(\beta(1\rightarrow3)\) glucans are more intense than in the TCY70(pYME) strain. Sterilization of the TCY70(pYME) strain causes a disappearance of mannan structures (912 and 971 cm\(^{-1}\) bands) as can be observed from the second derivative spectra (see Fig. 4c). Besides, bands at 999 cm\(^{-1}\), assigned to \(\beta(1\rightarrow6)\) glucans, and at 1042, 1080 and 1110 cm\(^{-1}\), attributed to \(\beta(1\rightarrow3)\) glucans [18] become more prominent. When the non-sterilized and sterilized AGLSC strains are compared, the second derivative profiles are quite similar except for the band at 1150 cm\(^{-1}\) (Fig. 4b) which appears as a small shoulder at 1147 cm\(^{-1}\) (Fig. 4d). This comparison also shows that the main glucan structures at 991 and 1079 cm\(^{-1}\) are unaffected and contribute to the preservation of the cell morphological forms. Another difference is the band at 1158 cm\(^{-1}\), also present in the non-sterilized and sterilized TCY(pYME) strains, and which appears more intense in the mutant strain (cf. Fig. 4d with a and c).

4. Discussion

When the TCY70 yeast strain was transformed by the p41F2 plasmid, cells became pink after 3 days culture on maltose medium. This intriguing phenotype appeared only after transformation of the TCY70 strain by the p41F2 plasmid. The resulting strain, AGLSC, possessed the same morphological and phenotypic characteristics as the wild-type strain TCY70. The difference between the two strains with respect to growth on maltose originated from the \(\alpha\)-glucosidase activity present in AGLSC (data not shown). This phenotype is not due to a mutation of \(ADE1\) or \(ADE2\) gene which gives a red color to cells [26]. In fact, when the AGLSC strain was cultured in minimal medium supplemented with adenine, no change was observed for the pink color, hence no phenotype reversion was noticed. Moreover, these strains grow on minimal medium without adenine.

FTIR spectra have been recorded for these strains and resolution enhancement methods such as curve-fitting and second derivative have been applied in order to follow the cell wall modifications that occurred upon transformation of the TCY70 strain. For this, the TCY(pYME) strain has been used as a negative control since it contained the plasmid pYME that had been used for constructing the p41F2 plasmid. From the spectral analysis based on curve-fitting methods, we observe that the mannans, which form peptidomannans at the level of the outer wall, are substantially diminished in the mutant AGLSC strain, as shown by the band areas at 806, 911, 971, and 1052 cm\(^{-1}\). This decrease can also be correlated with the loss in \(\alpha\)-helical and random structures that has been observed in the curve-fitted amide I and II protein bands (data not shown). Concerning \(\beta\)-glucan structures, which are the main components of the yeast inner cell wall, an increase in their content is seen in the mutant strain as indicated by the band areas at 894, and to a lesser extent that at 1103 cm\(^{-1}\). Another band at 879 cm\(^{-1}\) is downshifted to 873 cm\(^{-1}\) with an increase in intensity. This band is not assigned in the literature and we believe that it arises from a mixture of different \(\beta\)-glucans. The feature at 860 cm\(^{-1}\), absent in the TCY(pYME) strain, has a quite significant band area contribution. We do not know the exact origin of this structure yet, but since the mutant strain is deprived of peptidomannans and the cells are still viable, it is probable that they adapt to this deficiency either by the formation of new bonds or by a rearrangement of existing molecules.

FTIR spectroscopic investigation of the sterilized strains has confirmed the loss of mannans by this process. However, the appearance of the band at 1158 cm\(^{-1}\) in the sterilized AGLSC strain, already exists in the sterilized and non-sterilized TCY(pYME) is still unexplainable. Furthermore, there are no indications in our spectroscopic observations of the origin of the anomalous pink color. We strongly believe that it could originate from a carotenoid pigment, either present in quantities that are below the detection limit of the technique, or completely masked by the strong IR absorption of the cell constituents.

This study shows that FTIR spectroscopy affords a rapid and easy means of obtaining an indication of the nature of the major components of yeast cell wall. This method could be useful for the determination of differences in cell wall components from yeast mutants generated in the EUROFAN program, as well as for other biological or pharmacological applications.

References


