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Spectroscopic study of yeast *Saccharomyces cerevisiae* cell wall structure

Aurelijus Zimkus[1],

Audrius Misiūnas[2],

Larisa Chaustova[3]

[1] *Department of Biochemistry and Biophysics, Vilnius University, M. K. Čiurlionio 2, LT-01513 Vilnius, Lithuania*

[2] *Department of Organic Chemistry, Center for Physical Sciences and Technology, A. Goštauto 9, LT-01108 Vilnius, Lithuania*

[3] *Department of Bioelectrochemistry and Biospectroscopy, Institute of Biochemistry, Vilnius University, Mokslininkų 12, LT-08662 Vilnius, Lithuania*

The aim of this study was to evaluate the possibility of Fourier transform infrared (FT-IR) difference spectroscopy for the analysis of the cell wall structure of yeast *Saccharomyces cerevisiae* cells. For this purpose, the wild-type strain p63-DC5 and mutant XCY42-30-D(*mnn1*) strains, which possessed changes in cell wall polymer synthesis of the cell wall, were used. The spectra of wild and mutant strains differ in the region corresponding to polysaccharides. The intensity of vibration bands at 1 044 cm^{-1} demonstrated that the content of mannans was slightly increased in the mutant strain. At the same time, differences in the spectral range of vibrations 1 086–1 104 cm^{-1} showed that the mutant strain also had a higher content of β-(1–3) glucans. The results of the study showed that changes in the spectra were associated with yeast cell wall structure and demonstrated a high sensitivity and simplicity of FT-IR spectroscopy in the evaluation of yeast strains with a different cell wall architecture.

Key words: FT-IR, *Saccharomyces cerevisiae* cell wall structure, *mnn1* mutant

INTRODUCTION

In the traditional identification of yeast cell wall structure and composition, surface analysis tests such as enzymatic hydrolysis [1], spheroplasting rate assay [2], microscopy [3] and others are applied. However, these tests are limited by their long duration requiring a large quantity of cells and not available for all yeast strains. Moreover, these methods are rather rough and can damage the original structure of the polysaccharides in the wall.

Infrared spectroscopy is based on measuring the bond vibrations excited by radiation of a suitable frequency. Absorption of infrared light by cellular compounds results in a fingerprint-like spectrum that can be identified by comparison to reference spectra.

* Corresponding author. E-mail: aurelijus.zimkus@gf.vu.lt

FT-IR spectroscopy is a powerful technique for studying biological macromolecules and complex biological systems such as tissues and cells, for identifying and classifying microorganisms. FT-IR spectroscopy has been shown to be simple in use and very sensitive to small changes in the composition of cells, leading to the conclusion that the identification of yeasts at the strain level might be possible under well-controlled conditions [4–6].

It is important to note that infrared spectra do not only describe the composition of a cell, but also give additional information about molecular structure and conformation. The importance of FT-IR spectroscopy is that it allows real-time *in vivo* detection of dynamic interface events in near-physiological conditions [7–9].

Yeast *Saccharomyces cerevisiae* has a rigid cell wall outside its cell membrane. Yeast cell wall density, thickness and structure are the factors of major importance when inves-

tigating exogenous agent interaction. The layered structure of the cell wall, being a general phenomenon in yeast, modifies the surface properties such as hydrophobicity, electrical charge, sexual agglutinability, and porosity [10–13]. The wall of a yeast cell is a remarkably thick (100–200 nm) envelope, which contains some 15 to 25% of the dry mass of the cell. Major structural constituents of the cell wall are polysaccharides (80–90%), mainly glucans and mannans, with a minor percentage of chitin. Both β-(1–6) and β-(1–3)-linked glucans provide strength to the cell wall, forming a microfibrillar network. Mannans are presented as α-(1–6)-linked inner core with α-(1–2) – and α-(1–3)-side chains. Mannoproteins determine the cellular morphology and play a critical role in maintaining cell integrity during cell growth and division, under stress conditions, upon cell fusion in mating. The linkage between mannoproteins and β-(1–6)-glucan plays the central role in organizing the yeast cell wall [10–13].

The wild-type *S. cerevisiae* p63-DC5 strain with a wall structure of the classical description and the mutant XCY42-30D(*mnn1*) strain with a mutation involved in the glycosylation of mannoproteins were used. The mutant strain was defective in the formation of the α-(1–3)-mannosyl-transferase which is presumed to be involved in the addition of the terminal α-(1–3)-linked mannose unit to form the mannotetraose side chain [14, 15].

The aim of the study was to evaluate changes in *S. cerevisiae* XCY42-30D(*mnn1*) cell wall structure by applying FT-IR spectroscopy analysis.

EXPERIMENTAL

Infrared spectroscopy. FT-IR spectra were recorded at room temperature with a Perkin–Elmer Spectrum GX FT-IR spectrometer. The spectral resolution was set to 4 cm^{-1}, and all spectra were acquired by 200 scans. Samples of a concentrated yeast suspension ($2 \cdot 10^{10}$ cell/ml) were compacted between ZnSe windows and measured in the 950 cm^{-1} to 1 750 cm^{-1} region. Solvent spectra were normalized according to the association band of water at 2 125 cm^{-1} and subtracted from the yeast-containing sample spectra.

Yeast strains and cultivation. The following *Saccharomyces cerevisiae* strains were used in this study: p63-DC5 (*MATa, ade1, leu2–3, 112 his3*) and XCY42-30D (*MATα, ade2–101, adex, ura3, trp1, lys2, leu2–3,112,* Δ*mnn1::LEU2*). Yeast cells were grown in YPD (1% yeast extract, 2% peptone, 2% glucose) medium at 30 °C to OD_{590} 0.2–0.4 (exponential phase), washed with 10 mM Tris-HCl buffer, pH 8.0 and concentrated.

Statistical analysis. All spectral and data treatments were performed using LabSpec software (DILOR, Lille, France). The results presented in Fig. 3 are means ± standard errors of three experiments.

RESULTS AND DISCUSSION

The FT-IR spectroscopic analysis of *S. cerevisiae* p63-DC5 and XCY42-30D(*mnn1*) yeast cells was performed, and the wave numbers with assignments of the bands are presented in Table. Spectral assignments were done according to the literature data [16].

Figure 1 shows normalized FT-IR spectra of p63-DC5 and mutant XCY42-30D(*mnn1*) strains in the frequency range 950–1 750 cm^{-1}.

We were interested in the spectral vibration in the range 950–1 185 cm^{-1} in which differences in the spectral range of mannans and glucans are presented. Bands in this range are assigned to the C–OH, C–O–C and C–C glycosidic stretching vibrations of the pyranose rings of the carbohydrates [17, 18]. Vibrations of C–OH, C–O–C and C–C groups are in a very crowded spectral region between 1 000 and 1 160 cm^{-1} where symmetric PO_2 stretching vibrations appear. Since the content of glucan and mannan in the yeast cell is much higher than that of phosphate (Fig. 3), we suggest that vibrations in this spectral region represent carbohydrates [14, 19].

For more detailed information, differential spectra of wild p63-DC5 and mutant strains in the 990 to 1 185 cm^{-1} region are presented in Fig. 2c.

The characteristic band at 1 045 cm^{-1} demonstrates that the content of mannans is slightly increased in the XCY42-30D (*mnn1*) strain. At the same time, more intensive bands

Table. **Assignments of yeast FT-IR bands for *S. cerevisiae* p63-DC5 and mutant XCY42-30D(*mnn1*) strains corresponding to polysaccharides**

p63-DC5	XCY42-30D(*mnn1*)	Assignments
Wavenumber, cm^{-1}	Wavenumber, cm^{-1}	
970	970	Mannan band (C–O–C, C–C, and C–OH stretching of pyranose ring), O–P–O, and C–O–P stretching
1 027	1 028	β(1→4) glucan band (C–O–C, C–C, and C–OH stretching of pyranose ring), O–P–O, and C–O–P stretching
1 044	1 045	Mannan band (C–O–C, C–C, and C–OH stretching of pyranose ring), O–P–O, and C–O–P stretching
1 086	1 084	β(1→3) glucan band (C–O–C, C–C, and C–OH stretching of pyranose ring), O–P–O, and C–O–P stretching
1 104	1 106	β(1→3) glucan band (C–O–C, C–C, and C–OH stretching of pyranose ring), O–P–O, and C–O–P stretching
1 156	1 152	β(1→3) glucan (C–O–C, C–C, and C–OH stretching of pyranose ring), O–P–O, and C–O–P stretching

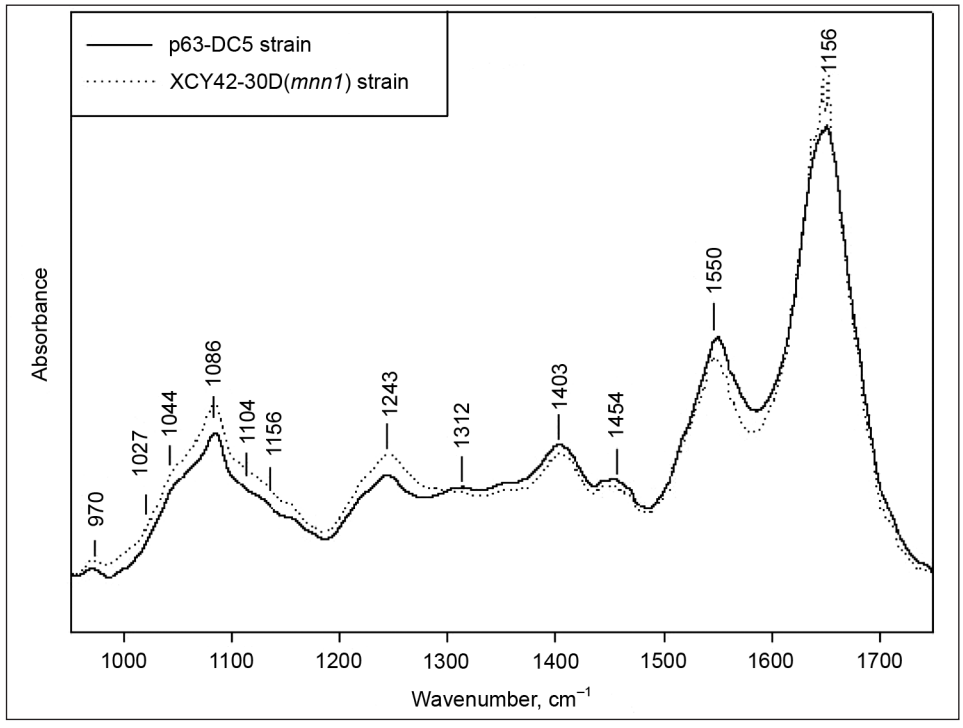

Fig. 1. FT-IR spectra of yeast *S. cerevisiae* p63-DC5 and XCY42-30D(*mnn1*) strains in the frequency region of 950–1 750 cm^{-1}

located at 1 086 cm^{-1}, 1 104 cm^{-1}, and 1 156 cm^{-1} showed that the mutant strain had a higher content of β-(1–3) glucans (Fig. 2, Table).

This seems surprising, because the mutant was defective in mannosyltransferase which is involved in the connection of the terminal mannose monomers to form the α-(1–3) linked mannotetraose side chain. Mannans with α-(1–2) linked mannoses are known to dominate in the cell wall of the mutant strain, which form side chains with at least two units (Fig. 3). On the contrary, the wild strain has a more branched mannan structure with three or more mannose units [14, 15].

We suppose that a decrease in the formation of α-(1–3) linked mannose in the mutant strain can be compensated

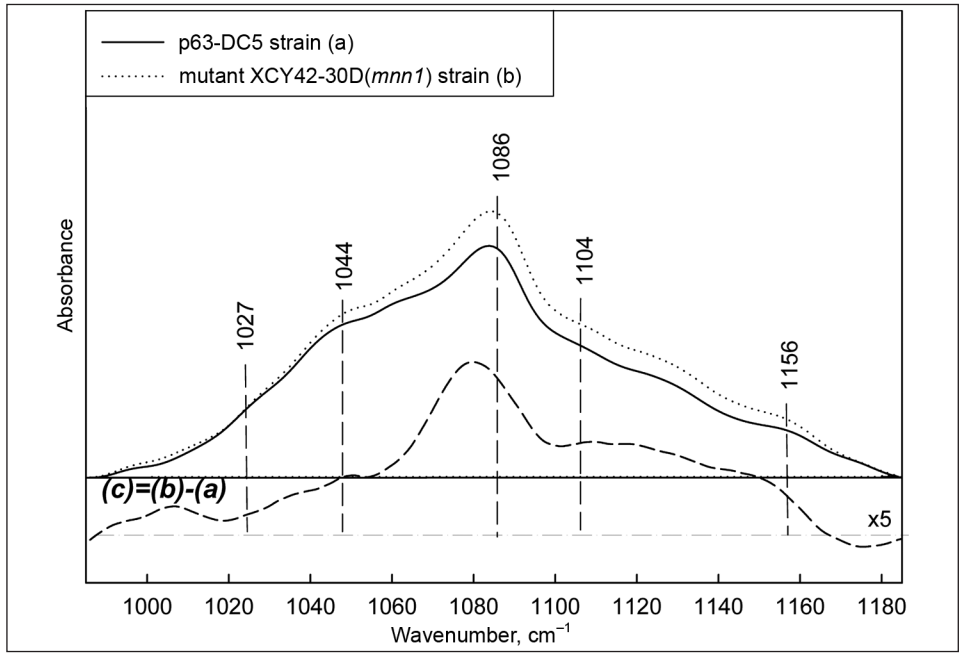

Fig. 2. FT-IR spectra of yeast *S. cerevisiae* p63-DC5 (*a*) and XCY42-30D(*mnn1*) (*b*) strains in the frequency region of 990–1 185 cm^{-1}. Difference spectrum (*c*) is shown. In subtraction procedure, spectra were normalized by the intensity of the 1 454 cm^{-1} peak

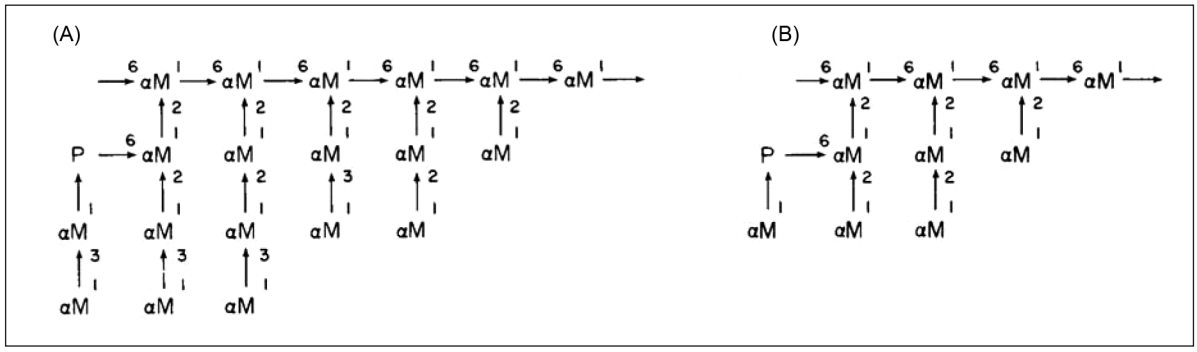

Fig. 3. Mannan polysaccharide chain structure of different yeast strains. *S. cerevisiae* p63-DC5 (A) and XCY42-30D(*mnn1*) (B)

by increasing the synthesis of α-(1–2) and α-(1–6) linked, less branched mannans, thus increasing the total content of mannans. At the same time, the decrease of α-(1–3) linked mannose in the mutant strain was additionally compensated by intensifying the synthesis of β-(1–3) linked glucans which determine cell wall rigidness and stability.

Our data are in agreement with data of De Nobel et al. who showed that the outer protein layer, the N- and O-linked side-chains of mannoproteins determine the properties of yeast cell wall. Mutations of the glycosylation and mannosylation pathways collectively affect all mannoproteins and result in multiple cell wall phenotypes, such as a compound or altered cell wall composition [20].

Our results have shown that changes in the cell wall of XCY42-30D strain influence growth rate (Fig. 4). The growth of mutant cells in YPD medium was slower than of the wild strain. After 180 min of incubation, the mutant strain reached the optical density of 0.3, whereas the wild strain reached 1.0. The slower growth of the mutant strain indicates that, although mannan is not essential for viability, survival without mannan is dependent on the cells able to sense changes in the cell wall structure [21].

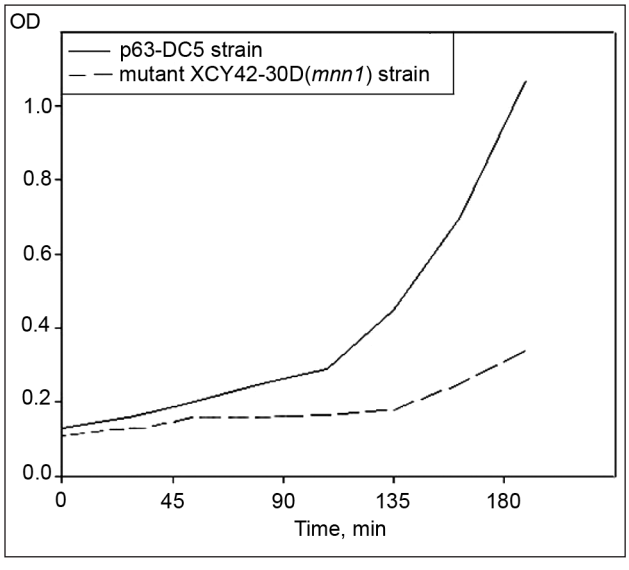

Fig. 4. Time course of cultivation of yeast *S. cerevisiae* p63-DC5 (a) and mutant XCY42-30D(*mnn1*) (b) strains. Overnight grown yeast cell culture was diluted to OD_{590} of 0.12–0.16 and allowed to grow further

CONCLUSIONS

This study shows that FT-IR spectroscopy is a rapid and informative method of obtaining information about the nature of the major components of yeast *S. cerevisiae* cell wall. We expect that this method could be useful for the determination of differences in cell wall compounds of various yeast strains and mutants.

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MIELIŲ *SACCHAROMYCES CEREVISIAE* LĄSTELIŲ SIENELĖS STRUKTŪROS TYRIMAI FT-IR SPEKTROSKOPIJOS METODU

S a n t r a u k a

Šio tyrimo tikslas buvo įvertinti Furje transformuotos infraraudonųjų spindulių spektroskopijos (FT-IR) tinkamumą mielių *Saccharomyces cerevisiae* ląstelės sienelės struktūros analizei. Šiuo tikslu buvo naudojami laukinis kamienas p63-DC5 bei mutantinis XCY42-30D (*mnn1*), kuris turi pokyčių ląstelės sienelės karbohidratinių komponentų sintezėje. Laukinio ir mutantinio kamienų spektrai skiriasi srityje, atitinkančioje polisacharidų absorbcijos sritį. Vibracinių juostų intensyvumo pakitimas 1044 cm^{-1} parodė, kad mutantinis kamienas turi nežymiai padidėjusį manano kiekį. Vibracijų juostų intensyvumo pokyčiai 1086–1104 cm^{-1} bangų intervale parodė, kad mutantinis kamienas turėjo daugiau β-(1–3) gliukano. Gauti duomenys rodo, kad spektrų pokyčiai, kuriuos pavyko užregistruoti tiriant ląsteles FT-IR spektroskopijos metodu, atspindi tirtų mielių kamienų sienelių struktūrinius ypatumus. Daroma išvada, kad FT-IR spektroskopijos metodas yra tinkamas mielių ląstelių sienelių kompozicijai įvertinti.