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Running Title: Process effects on cell wall composition and nanomechanical properties

Keywords: Cell wall, autolysis, atomic force microscopy, β-glucans, chitin, yeast

Abstract

The *Saccharomyces cerevisiae* cell surface is endowed with several technological properties, among which its effective biosorption capacity to reduce undesirable molecules, such as volatiles phenols in wine or mycotoxins in food. Since cell surface properties are intimately linked to cell wall structure, the aim of this study was to investigate effects of autolyzing/drying process on the biochemical composition of cell wall and on the nanomechanical properties of cell surface. To achieve these goals, we used a recently developed method to analyze the biochemical composition, and we took advantage of Atomic Force Microscopy to access the nanomechanical properties. We choose to work on two industrial strains because of their difference in winemaking applications and sorption properties. We found that the autolysis/drying process of the two strains did not significantly modify the biochemical composition of their cell wall. It, however, caused severe changes in cell surface topography characterized by a 4-fold increase of the roughness and by a global increase in adhesion characteristics of the autolyzed/dried samples. Though this process had some effects on biomechanical properties, the two strains natively harbored differences in biophysical properties that could be accounted by difference in cell wall composition, as the strain with the highest mannoproteins content was also characterized by the presence of highly adhesive patches forming nanodomains,. Comparative transcriptome analysis uncovered a strong upregulation of flocculin encoding *FLO11* gene in this industrial strain that corroborated with higher interaction to concanavalin A-functionalized AFM tip, leading to the idea that this protein is responsible for the patches formation.

1. Introduction

The yeast *Saccharomyces cerevisiae* is used since millennia in traditional biotechnological purposes, such as the production of wine, beer and bread. In addition to this role in fermented foods and beverages, this yeast species is attracting increased attention because of other relevant biological properties, such as antimicrobial and biosorption activities, that make them promising candidates for a wide range of applications not limited to the food sector. Indeed, the antimicrobial activities against undesirable bacteria and mold have been recognized since a long time to provide probiotic properties of yeasts, although the mechanisms underlying these antagonistic activities remain unclear {Hatoum, 2012 5788 /id}. Biosorption is a cell surface property that is defined as the physicochemical process wherein undesirable molecules which can be a toxin, heavy metals or volatile compounds interact and accumulate at the surface of microbial cells {Petruzzi, 2014 5810 /id;Fomina, 2014 5809 /id}. Since this property is displayed by inactive or dead cells and does not require intracellular energy, the physicochemical properties of the microorganism's surface are determining factor in the nature of the interaction and in the sorption capacity. The surface of yeast cell corresponds to a thick wall of about 120-180 nm, exhibiting an ultrastructure of two distinct layers as visualized by electron microscopy {Osumi, 1998 235 /id}: an outer layer mainly constituted of mannoproteins (30-50% of cell wall dry mass) and an inner layer made of chitin (2 - 5 % of cell wall dry mass) and β-glucans (40 - 60 % of cell wall dry mass). Extensive biochemical analysis allowed to propose a supramolecular structure of the mature cell wall in which the chains of β-(1,3) glucose residues are branched to β-(1,6)-glucans, forming a fibrillar network, that serves as backbone to which are linked chitin, β-(1,6)-glucan and mannoproteins. In addition, the outer layer of mannoproteins can be linked to the inner layer through β-(1,6)-glucan via a remnant of a GPI anchor {Lesage, 2006 3212 /id}. It is therefore suggested that the sorption capacity for a given molecule can be linked to a specific component of the yeast cell wall. This suggestion has been verified in very few cases. For example, a major role of mannoproteins in the retention of volatile aroma by yeast cell walls has been reported {Lubbers, 1994 551 /id;Pradelles, 2008 5792 /id}, whereas β-glucan was

showed as a valuable microbiological binder of the mycotoxin zearalenone {Yiannikouris, 2004 3122 /id}. However, these studies were mostly carried out with pure fractions of polysaccharides and did take into account neither the complexity and variability of the cell wall composition, that can depend on culture conditions {Aguilar-Uscanga, 2003 1148 /id} and strains background {Nguyen, 1998 432 /id}, nor the process by which yeast cells are prepared and used as biosorbant. In this context, Pradelles *et al* {Pradelles, 2008 5792 /id} investigated effects of cell wall composition on the sorption of the undesirable 4-ethylphenol. While they confirmed a predominant role of mannoproteins in the sorption capacity of this volatile aromatic compound, they also highlighted the fact that drying the yeast biomass greatly increased the sorption capacity of this molecule, which was in part correlated with increased surface hydrophobicity of the dried biomass rehydrated subsequently in water {Pradelles, 2009 5793 /id}. Taken together, these results indicate that the drying process and more generally the method to prepare yeast cell wall fractions for biosorption applications may have a strong impact on physico-chemical properties of the cell surface. This question is particularly pertinent when we consider that yeast cells employed for biosorption applications are obtained from cultures that are autolysed and air-dried at 55°C, and commercially supplied as 'yeast cell wall' fraction or YCW. Autolysis is a term that describes the breakdown of cell constituents by action of endogenous enzymes. It can occur naturally when yeast have completed their growth cycle and entered the death phase, or it can be induced at high temperature and low pH {White, 2002 4963 /id} {Martinez-Rodriguez, 2009 455 /id}. Proteases, β-glucanases and chitinases are among autolytic hydrolases that are implicated in this process, and therefore, it can be expected that this process may cause important change in cell wall composition. Accordingly, Martinez-Rodriguez *et al* {Martinez-Rodriguez, 2001 4964 /id} reported important ultrastructural changes during the autolysis of yeast cells using Low Temperature Scanning Electron Microscopy (LTSEM), whereas Aerosol Flow Tube - Fourier Transform Infrared Spectroscopy (AFT-FTIR) that was used to monitor global biochemical changes during autolysis revealed hydrolysis of mannans and β-glucans. In spite of these apparent hydrolyses, microscopic observation of autolyzed cells showed that the cell shape was fully

retained suggesting that the cell wall structure was not destroyed {Hernawan, 1995 4965 /id}. Altogether, these studies raised fundamental questions about the effects of industrial processes on structure and properties of yeast cell surface.

These questions can be addressed using Atomic Force Microscope (AFM) which is a very powerful force microscope technology allowing tridimensional images of cell surface and quantitative measurement of nanomechanical properties such as roughness and elasticity (Young modulus) {Dague, 2007 443 /id;Dufrene, 2010 5053 /id}. We used recently this technology to image and quantify surface properties of yeast cell wall mutants {Dague, 2010 5052 /id}, to investigate effects of antifungal drug caspofungin and heat shock on yeast cells (Formosa et al., 2013; Pillet et al., 2014). These studies led to the finding that the nanomechanical properties of the cell surface are merely dependent on the molecular architecture of the cell wall and not on a specific polysaccharide component of the wall. To gain deeper knowledge on the molecular organization of cell wall, the single molecule force spectroscopy (SMFS) can be employed. This method consists in probing the cell with an AFM tip that is functionalized with a specific ligand that could interact with some proteins present at the cell surface {Hinterdorfer, 2012 463 /id}. Accordingly, probing the yeast cell surface with AFM tip functionalized with concanavalin A (conA tip), which is a lectin protein that interacts with D-mannose and glucose residues (ref), has provided quantitative information about distribution, frequency of adhesion events, flexibility and extension of mannans on the surface of different yeast strains {Dufrene, 2013 459 /id}. With this approach, Alsteens *et al*. {Alsteens, 2008 457 /id} investigated the surface properties of a bottom-fermenting and a top-fermenting yeast. They showed that the latter species has higher adhesion frequency and more extended polysaccharides than the former one, consistent with the fact that the surface of top-fermenting yeast cells is richer in proteins and more hydrophobic.

The purpose of this study was therefore to investigate effects of industrial autolysis and drying process on cell wall composition and cell surface nanomechanical properties of two industrial yeast strains that bear differences in winemaking properties. Effects of this process on adhesive

properties and on polysaccharides distribution using conA tip of the two strains were also analyzed by comparing whole active-living from rehydrated dried yeast cells.

2. Material and Methods

2.1. Strains and culture conditions

Two diploid industrial winemaking *Saccharomyces cerevisiae* strains, L71 and L69, from Lallemand Inc. (Blagnac, France) were studied. Differences between the two strain was mainly that the L69 strain has a higher mannoproteins content, expresses different sensorial notes during wine fermentation and presents good capacity to interact with volatile undesirable phenols (Lallemand Inc., unpublished data). The strains were propagated under standard laboratory conditions, which corresponded to growth in 200 ml YPD medium (1% [w/v] yeast extract, 2% [w/v] bactopeptone and 2% [w/v] glucose) in 1 liter shake flasks at 30°C under shaking at 200 rpm. They were also propagated under industrial standard protocol, involving batch and fed-batch growth on molasses based medium, collected, and concentrated to produce packed yeast cells termed 'cream'. The latter cream was then subjected to autolysis (20 hr at 55°C), followed by separation and then by spray-drying to produce the dry "YCW" (industrial term meaning Yeast Cell Wall fraction that is used for this type of yeast packaging). This autolytic/drying process leads to > 99.9 % of cell mortality as estimated by methylene blue coloration test {Cot, 2007 5468 /id}. YCW were provided in 30 g packets sealed under vacuum and stored unopened at 4°C until required. Rehydration was performed according to the manufacturer's guidelines (Lallemand Inc., Blagnac, France): 0.1 g of YCW was sprinkled onto 10× its weight of temperature equilibrated (30°C) sterile water in a tube. The YCW was left to absorb water slowly for 20 min, mixed gently to form slurry and the temperature was maintained at 30°C throughout rehydration.

2.2. AFM measurements

2.2.1. Sample preparation

Cells collected from exponential growth in YPD or after rehydration of YCW were washed two times in sodium acetate (18 mM CH_3COONa, 1 mM $CaCl_2$ and, 1 mM $MnCl_2$, pH 5.2), and immobilized on polydimethylsiloxane (PDMS) stamps prepared as described in {Dague, 2011 442 /id}. Briefly, freshly oxygen activated microstructured PDMS stamps were covered by 100 µL of yeasts sample at

148 OD_{600} around 1.0. The cells were then deposited into the microstructures of the stamp by
149 convective/capillary assembly.

150 *2.2.2. AFM imaging*

151 Images and force-distance curves were recorded at room temperature in acetate solution
152 using an AFM Nanowizard III (JPK Instruments, Berlin, Germany) and MLCT AUWH cantilevers
153 (Bruker, Santa Barbara, USA). The spring constants of the cantilevers were systematically measured
154 by the thermal noise method according to {Hutter JL, 1993 5529 /id} and were found to be in the
155 range of 0.01-0.02 $N.m^{-1}$. AFM height and adhesion images were recorded in Quantitative Imaging™
156 mode {Chopinet, 2013 454 /id}, and the maximal force applied to the cell was limited to 1.5 nN.

157 *2.2.3. Surface roughness measurements*

158 To measure cell surface roughness, images were obtained by scanning at high resolution
159 (1µm x 1µm areas) on independent cell samples (at least 5) in the contact mode. In contact mode,
160 the tip is in contact with the surface of the sample and scans this surface horizontally with a constant
161 force, which is lower as possible. Height and vertical deflection images were both recorded with the
162 contact mode. Height images with the same center of offset were processed and analyzed with the
163 power spectral density method (JPK data processing software) consisting of average roughness (Ra in
164 nm) measurements on five boxes of five different sizes for each images.

165 *2.2.4. Stiffness measurements*

166 Results were analyzed using the Data Processing software from JPK Instruments. The stiffness
167 value (k_{cell} in $N.m^{-1}$) measured on cells was determined from the cantilever spring constant (k) and the
168 slope (s) of the linear part of the force curve according to {Arnoldi, 2000 4684 /id}:

$$kcell = k\left(\frac{s}{1-s}\right)$$

169 *2.2.5. Force spectroscopy*

170 For force spectroscopy experiments, the applied force was kept constant at 0.5 nN. To probe
171 cell surface polysaccharides, AFM tips were functionalized with the concanavalin A (ConA) from
172 *Canavalia ensiformis* (Sigma-Aldrich, L7647) via a dendritip as described in {Jauvert, 2012 5569 /id}.
173 The coupling with the lectin was made by immersion of the dendritip in 100 µL of ConA solution (100

µg.ml^{-1} in a 0.1 M carbonate buffer). After 1 hr incubation, 100 µL of $NaBH_4$ 3.5 mg.mL^{-1} solution was added and incubated 15 min in order to reduce the unreacted groups. Finally, the cantilever bearing the functionalized tip was washed three times and stored in acetate buffer. To analyze the stretching of polysaccharides at the surface of the cell, elongation forces were stretched using the worm-like chain (WLC) model introduced by Bustamante {Bustamante, 1994 5811 /id} which describes the polymer as a curved filament and the force F *vs* the extension x is given by:

$$F(x) = k_bT/l_p\,[0.25\,(1-x/L_c)^{-2} + x/L_c - 0.25]$$

where the persistent length (l_p) represents ~~the stiffness of the molecule~~, the contour length (L_c) is the total length of the stretched molecule, k_b is the Boltzmann constant and T is the absolute temperature. This model has already been successfully used for the stretching of polysaccharides of yeasts especially for *S. cerevisiae* and *S. carlsbergensis* {Alsteens, 2008 457 /id} and gives the best fitting of force curves. Blocking control experiments were performed by injecting 100 mM D-mannose solution into the cell surface.

2.3. Cell wall isolation and quantification of polysaccharides

Yeast cell walls were extracted and purified from yeast culture of L71 and 69 taken in exponential phase of growth on glucose (OD_{600}=1), rehydrated YCW and 'cream' according to the protocol described in {Francois, 2006 4678 /id}. Cell wall polysaccharides mannan, chitin, β-(1,3) and β-(1,6) glucan in the purified cell walls were determined by a combination of acid and enzymatic hydrolysis recently developed {Schiavone, 2014 457 /id} and quantification of the release sugar monomers (mannose, glucose and *N*-acetylglucosamine) was determined by High Performance Anionic Exchange Chromatography (HPAEC) with Pulsed Amperometric Detection (PAD) as described in {Dallies, 1998 224 /id}.

2.4. Transcriptomic analyses

Three independent biological cultures of industrial strains L71 and L69 were carried out in 50 ml of YPD in a 250 ml shake flasks. Yeast cells (about 10 OD_{600} units) were collected at OD_{600}=1 by

centrifugation (3,000 rpm, 4°C, 2 min), followed by a washing step with 1 ml of sterilized water. The cell pellets were immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction. RNA extraction, quantification and labelling were carried out as described in {Alkim, 2013 5685 /id}. Labeled cDNA were hybridized on Agilent glass slides microarrays, which bear the whole *Saccharomyces cerevisiae* genome (see details at http://www.biocompare.com/ProductDetails/760330/S-cerevisiae-Saccharomycescerevisiae-Whole-Genome.html). Hybridization was carried out in an automatic hybridization chamber (Agilent Technologies, Wilmington, USA) for 17 hr at 65°C. The hybridization signals were detected by scanning using Innoscan 900 laser Scanner (Innopsys Instruments), and transformed to numerical values using Feature Extraction V.11.5.1.1. The microarrays hybridization and processing were carried out at the Biochips Platform of Toulouse (http://biopuce.insa-toulouse.fr).

Transcriptome analyses were done in R computing environment (www.R-project.org) using the Limma package {Yang, 2002 2877 /id} (www.bioconductor.org). The estimates used for the foreground and background intensities were the median of pixels intensity. Raw data were imported into R and spot quality weights were performed assigning a weight of 1 or 0 to each spot. Low-quality spots, non-uniform spots, spots with low signal/background ratio or spots with low signal-to-noise ratio and empty or non-validated spots were down weighted. Data were preprocessed by base 2 logarithmic transformation and within-array normalized was performed using the weighted global median (spots with zero weight were not included in the normalization). To achieve consistency of expression values between arrays, normalization across all the microarrays for each strain was performed. After normalization, the expression of a gene was calculated by the median of replicate spots within each microarray. Gene expression data for both strains were pairwise compared using the Limma package {Smith, 2005 5083 /id}. Genes with significant evidence for differential expression were identified with a modified *t*-test in conjunction with an empirical Bayes method to moderate the standard errors of the estimated log-fold changes. The *P*-values for the genes of interest were adjusted for multiple testing by the ''BH'' method {Hochberg, 1990 3432 /id}.

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3. Results

3.1. Effects of autolysis/drying process on cell wall composition of two industrial yeast strains.

Since it is considered that the interaction cell-environment and sorption capacity to undesirable compounds are dependent on the surface properties of yeasts, which are in turn impacted by the biochemical composition of cell wall {Pradelles, 2008 5792 /id;Nunez, 2008 4953 /id;Armando, 2012 5219 /id}, we aimed at investigating effects of the industrial autolysis/drying process on cell wall composition of two industrial strains. These strains were chosen on the basis of their different winemaking behavior and content of mannoproteins (Lallemand Inc, unpublished data). To evaluate impact of the process, we compared the polysaccharides composition of cell walls purified from autolyzed/dried yeast cells, which are commercialized as "YCW", with those extracted from the same industrial strains that were cultivated under laboratory standard conditions (Yeast Peptone Dextrose medium) and harvested at the exponential phase of growth. The cell wall composition was also determined on packed yeast cells which are provided as 'cream' and which was obtained from batch fermentation in molasses-based medium. Results of this analysis are reported in Table 1. Overall, the polysaccharides content in purified cell wall was between 85 to 90 % of the cell wall dry mass, the remaining 10 to 15 % corresponded to proteins (data not shown). Also, the proportion of β-(1,3), β-(1,6)-glucan, mannan and chitin in cell wall of the two industrial strains were statistically not different (p value > 0.05) between the three conditions/ treatments investigated. However, it can be noticed a strain effect on the biochemical composition of the cell wall that was statistically significant. Indeed, strain L71 either in a living, cream or dried stated exhibited two times more chitin (p value < 0.001) and a higher proportion of β-(1,6)-glucan in total β-glucan than in strain L69. On the other hand, the mannan content of the L69 strain was roughly 20 -25 % higher than in strain L71 (p value < 0.01), which agreed with the fact that this industrial winemaking strain was selected on the basis of its higher production of mannoproteins. This high mannan content of strain L69 could be associated with the fact that this strain has a higher surface hydrophobicity than strain

L71 (data not shown) as well as to a tendency to hold cells together in small branched chain during growth and to produce clumps upon rehydration of YCW produced from this strain (data not shown).

3.2. Effects of autolysis/drying process on nanomechanical properties of two industrial yeast strains.

In accordance with a previous work {Hernawan, 1995 4965 /id}, rehydrated YCW regained round to ovoid cell shape like a normal living yeast cell (data not shown). This cell shape retention allowed using our immobilization methodology that is based on trapping of single round/ovoid yeast cell into microchambers made in microstructured PDMS stamp by capillary/convective means {Dague, 2011 442 /id}. This methodology is very efficient to readily monitor several isolated cells in liquid environment by AFM. Topographic images using Quantitative Imaging™ mode {Chopinet, 2013 454 /id} of living yeast cells from strain L71 and rehydrated YCW derived from L71 strain is reported in Figure 1 (a and b). For both types of yeast samples, height images illustrated a rather round shape of the immobilized object with an apparent smooth surface. However, a closer inspection of the AFM images from the rehydrated sample revealed the presence of a single hole of 600 nm diameter size curiously localized in the middle of immobilized cell (Fig. S1 in supplementary data), and this feature was obtained in at least 15 % of the analyzed cells. At higher resolution, height images (Fig.1 c and d) from contact mode clearly showed significant differences of the surface between living and rehydrated sample from L71 strain, as the surface of the latter was severely fractured. In quantitative terms, the roughness of cell surface (determined on 1 µm x 1 µm areas) was around 1.9 ± 0.2 nm for the living cell and increased to 5.1 ± 1.1 nm for the rehydrated YCW sample (Table 2). This surface change could be linked to the ultrastructural changes that was reported by Low Temperature Scanning Electron Microscopy (LSTEM) of a commercial yeast strain after 14 hr of induced autolysis in a model wine medium {Martinez-Rodriguez, 2001 4964 /id}. Figure 1 (e, f) shows adhesion images of a living and rehydrated cell from YCW of L71 strain that were obtained by probing the surface with an AFM tip in QI™ mode. As the tip is made of silicon nitride and the immobilized sample is under an acidic pH (sodium acetate 18 mM at pH 5.2), these conditions most likely favor hydrophobic

interactions, which are however relatively weak with both type of cells, and hence agreed with exposure of 'neutral' cell wall carbohydrates.

Similar AFM analyses were carried out with active living yeast cells and rehydrated YCW of L69 strain. The topographic images taken at low resolution (Fig 2, a and c) did not show difference in both types of cell samples immobilized in PDMS (Fig. 2). At higher resolution, some discretional dots could be visualized that were rather larger in rehydrated YCW (Fig 2, b and d). In addition, we found a hole in immobilized rehydrated samples of YCW in about at least 15 % of the AFM images, like it was seen for strain L71 (see Fig.S1 supplementary data). The force curves acquired in QI mode were also analyzed to obtain adhesion images. This analysis revealed adhesives patches that were not present in L71 strain and which became apparently bigger in the sample from rehydrated YCW (compared Fig. 2 a, e, i for active yeast cells with c, g and k for rehydrated YCW). On average, the patches from the rehydrated YCW had a mean area of 156663.4 ± 3904.4 nm^2 (0.016 ± 0.004 μm^2) and a diameter of 141 ± 32 nm (measured from 56 patches on 7 different cells with Image J software). Additional details of the morphology and mechanical properties of these patches are reported in Figure 3 and Figure S2 in supplementary data. From a cross-section taken through several patches (see the green dashed line), the corresponding height, adhesion and stiffness values were determined. This detailed AFM analyses showed that these patches corresponded to small protuberances exceeding the cell surface as shown in Figure 3Ae (also shown in Figure S2 b and d, in supplementary data).~~by about 30 - 40 nm (Fig. 3A).~~ These protuberances strongly interacted with the AFM tip (Fig. 3B). Example of adhesion force is schematically illustrated by force-distance curves taken from 3 different patches (Fig. 3B). On the contrary, these patches exhibited lower stiffness than the ground surface of the rehydrated YCW sample. Recently, similar observation was made with other *Candida albicans* cells that also harbored nanodomains at its cell surface represented by hydrophobic patches that were likely due to high expression the *ALS1* encoding cell surface protein (Alsteens et al., 2010; Formosa *et al.,* 2014, Nanomedicine). Taken together, these results provide some clues about the surface properties of L69 strain which were distinct from those of L71 strain.

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305 **3.3. Probing mannans polysaccharides at the cell surface using an AFM tip functionalized with**
306 **Concanavalin A.**

307 To go deeper in the analysis of the process effect on cell surface properties and illustrate
308 differences in cell surface between the two industrial strains, we employed the single molecule force
309 spectroscopy (SMFS) using an AFM tip functionalized with ConA. This molecule is a lectin protein that
310 has high affinity to α-mannosyl residues, with an unbinding force which corresponds to the rupture
311 of a single lectin-mannose has been estimated in the range of 60 pN {Alsteens, 2008 457 /id}. Figure
312 4 shows results of distribution, adhesion and flexibility of mannans at the surface of a living and YCW
313 rehydrated from L71 strain. Adhesion force maps (Fig. 4, b and f) obtained with 1024 force-distance
314 curves recorded with the ConA tip showed a relatively homogenous distribution of mannans
315 polysaccharides across the surface of the rehydrated L71 sample, whereas this distribution was
316 apparently less regular for the living cell. However, the adhesion frequency which represent the
317 percentage of single or multiple unbinding forces was in the range of 25 % for both types of yeast
318 samples (Fig. 4, c and g) with a mean value of unbinding forces around a maximum of 60 pN (58 ± 21
319 pN for the living cell and 65 ± 38 pN for the rehydrated sample, this difference being statistically not
320 significant with a calculated *p* value > 0.05) in accordance with a previous report {Alsteens, 2008 457
321 /id}. Moreover, this binding interaction was blocked at 92.5 % by addition of excess of mannose
322 solution onto the immobilized samples (see Fig.S2, in supplementary data). This result indicated that
323 the adhesion force merely originates from specific lectin-mannans interactions. However, the 7.5%
324 residual adhesion suggested some unspecific interactions. We also noticed that bud scars in both
325 type of yeast samples were not adhesive (data not shown). An additional property of the mannan
326 polysaccharides that can be obtained using this SMFS approach is the rupture distances which define
327 the distance needed to retract the conA tip from its binding with the mannan polysaccharides. These
328 data can be informative on the flexibility and extension of these macromolecules. As can be seen in
329 Fig. 4 (d and h), the rupture distances recorded for both types of samples was comprised between 0

330 and 250 nm, with a slight wider distribution towards larger lengths for the rehydrated sample
331 suggesting that the polysaccharides chains in this sample were slightly more adhesive and extended
332 than in living cells.

333 We then carried out a similar experiment with strain L69. Results of this SMFS analysis using
334 ConA tip are reported in Figure 5. It can be seen that, like for the previous L71 strain, adhesion force
335 map from the rehydrated YCW of L69 strain was more homogeneous than that of a living cell.
336 However, longer rupture distances (up to 350 nm) were recorded on the living cell of L69 strain as
337 compared to those with living L71 cells (compare Fig. 4d and Fig. 5d), suggesting difference in the
338 stretching of polysaccharides between these two strains. In addition, longer rupture distances in L69
339 than in L71 strain were consistent with higher adhesion and presence of patches at the cell surface of
340 the L69 strain. Alternatively or complementary to this explanation, it is possible that L69 strain
341 expressed more and/or different mannoproteins than L71 strain. Also, on average, the rupture
342 distance obtained with living cell of L69 was apparently longer than that of the rehydrated YCW of
343 the same strain (Fig.5 d and h).

344 **3.4. Transcriptomic analyses to infer differences in cell surface properties between the two**
345 **industrial strains.**

346 To seek for a molecular explanation of the difference between cell surface properties
347 between the two industrial strains, we carried out a genome-wide scale expression analysis using
348 DNA microarrays. On a global view, we obtained 392 differentially expressed genes between L69
349 versus L71 strain, that were distributed into 175 upregulated and 217 downregulated genes (see
350 table S1 and S2 in supplementary material). Major differences between L69 versus L71 strains were
351 found in the downregulation of several genes implicated in sulfate and methionine metabolism as
352 well as in a relatively increased expression of genes with yet unclear function. We looked more
353 carefully to genes implicated in cell wall biogenesis and assembly to find out potential differentially
354 expressed genes that could be linked to difference in cell surface properties between the two strains.
355 As reported in Table 2, strain L69 was characterized by higher expression levels of genes encoding

mannoproteins. In particular, *FLO11* encoding a mucin-like protein that belongs to the flocculation gene family {Lo, 1996 164 /id;Van Mulders, 2009 5797 /id} was strongly upregulated in L69 strain. Since this protein has a highly hydrophobic character {Karunanithi, 2010 5795 /id}, this can explain the hydrophobic adhesions recorded by AFM (Fig. 3). In addition, the relative higher expression of *YHR213w* encoding a putative flocculin may further contribute to this hydrophobicity property as well as to the presence of patches at the cell surface of L69 strain. There was however some cell wall encoding genes whose expression was slightly more expressed in strain L71 relative to L69 strain, such as *FLO5* encoding a lectin-like cell wall protein, but this differential expression can be considered as insignificant to account for difference in adhesion properties between the two strains.

4. Discussion

Combination of biochemical analyses and AFM technology allows us to observe that packaging yeast cells as "YCW" by autolysis and drying process significantly affected the global surface properties of the cells, in spite of the fact that the biochemical composition of the cell wall was barely altered by this packaging process. This result reinforces our previous finding showing no direct relationship between the polysaccharides composition of cell wall and its bionanomechanical properties {Dague, 2010 5052 /id;Pillet, 2014 5726 /id;Formosa, 2013 5624 /id}. Also, the finding that cell wall composition remained barely unchanged after autolysis and drying process is at variance to other reports that indicated some loss of β-glucan and mannans during this process {Cavagna, 2010 4950 /id;Giovani, 2007 4954 /id}. However, the discrepancy can be explained by the difference in the autolysis process. In our study, it is an industrial process that consisted in an induced autolysis at high temperature for 20 h, whereas other studies were related to yeast autolysis during wine fermentation that lasted several days and was done at lower temperature {Martinez-Rodriguez, 2009 455 /id;Alexandre, 2006 456 /id}. As compared to living or active yeast cells, the main effect of autolysis/drying process was to increase the roughness of the cell surface, which was visualized as a change from a relatively smooth to wrinkled structure. It remains to evaluate whether this modification has some impact in the sorption capacity of the yeast cells.

In a previous work, we showed that the mode of cultivation and medium composition influenced the cell wall composition {Aguilar-Uscanga, 2003 1148 /id}, while other studies demonstrated differences in cell wall composition among different yeasts species {Nguyen, 1998 432 /id}. Here, we furthermore showed that even for a same yeast species that is endowed with same genome, the biochemical composition of the cell wall and its cell surface organization can be dramatically different. These differences can be explained in part by difference in gene expression levels. Indeed, we found that the transcript levels of *FLO11* encoding a flocculin and of *YHR213w* that is suggested to code also for a flocculin, were relatively much higher in L69 than in L71 strain. In addition, several patches in the nanometer size were detected at the cell surface of L69 strain by AFM that resembled nanodomains identified at the cell surface of the pathogenic yeast *C. albicans* (Alsteens et al., 2010; Formosa et al., 2014). In *C. albicans*, these nanodomains were attributed to the presence of massive amount of *ALS1* encoding adhesins which forms amyloid due to seven residues sequence enriched in β-branched amino acids (Ile, Thre, Val) that are predicted to form intramolecular β-sheet like interactions or amyloids {Lipke, 2012 449 /id}. Recently, it was found that the sequence of the *S. cerevisiae* Flo11 protein presents such amyloid forming motifs VVSTTV and VTTAVT that can lead to partial β-aggregation {Ramsook, 2010 5812 /id}. Therefore, the higher expression for this flocculin encoding gene in L69 strain may account for the formation of the nanodomains, for the difference in the stretching properties of the mannoprotein of this strain as compared to L71 strain, as well as for the tendency of L69 cells to form aggregates (data not shown). Furthermore, the higher content of mannans measured in L69 strain can be also explained by a higher level in Flo11 which is known to be a very highly glycosylated protein as well as by other GPI-anchored mannoproteins, since the expression of their corresponding genes was found increased as compared to strain L71. On the other side, the reduced levels of β-(1,6) glucan and chitin in L69 strain were not associated with reduced expression levels of the main genes related to their biosynthesis or regulation {Lesage, 2006 3212 /id}, suggesting that these differences between the two strains would be at the post-transcriptional regulation. For chitin, it is well established that chitin synthase 3 (CSIII)

encoded by *CHS3* is responsible for > 90 % of the chitin in exponential growing yeast and that this protein is regulated at the post-translational level, which implicated notably an endocytic process mechanism that retrieves CSIII from chitosomes to plasma membrane (review in {Orlean, 2012 5610 /id}). Regulation of β-(1,6)-glucan is also very complex and takes place along the secretory pathway because several of the proteins implicated in the synthesis of this polymer were localized in the ER, Golgi or plasma membrane. Thus, the synthesis of β-glucan can be regulated at the level of any of these enzymatic steps during this process.

In conclusion, our combined biochemical and biophysical analyses of living-active and autolyzed cells confirmed, on the one hand that for a given yeast strain, changes in biomechanical properties of the cell surface caused by the process packaging are not linked to changes in cell wall composition. On the other hand, the significant difference in a particular and specific cell wall component between yeast strains could result in important difference in the biophysical properties of the surface between these strains.

5. Acknowledgements

We thank Henri Durand and Anne Ortiz-Julien from Lallemand Inc. for critical discussions during this work. This work was supported in part by a grant n°10051296 from Region Midi Pyrénées to JMF and an ANR Young Scientist project ANR-11-JSV5-001-01, n° (SD) 30 02 43 31 to ED. ED is researcher at the Centre National de la Recherche Scientifique (CNRS) and MS is supported by a grant from Lallemand SAS (Cifre fellowship).

6. References

7. Legend to figures:

Figure 1: Cell surface topography of a living cell and rehydrated sample from strain L71

AFM height (a, b), contact (c,d) and adhesion images (e, f) of a living (a, c, e) and a rehydrated YCW sample (b, d, f) of YCW from strain L71. Living cells were from L71 cultivated exponentially on YPD medium whereas the rehydrated sample was from autolysed/dried sample of L71 obtained as "YCW" and rehydrated according to manufacturer's recommendation as described in Material & Methods.

Figure 2: Cell surface topography of a living cell and rehydrated cell from strain L69

AFM height (a, b), adhesion (c, d) and stiffness (e, f) images of a living (a, b, c) and a rehydrated YCW sample (d, e, f) from the strain L69. The living cell was from an exponential culture of L69 strain on YPD medium, whereas rehydrated cell was from autolysed/dried sample of L69 obtained as "YCW" and rehydrated according to manufacturer's recommendation as described in Material & Methods.

Figure 3: Detailed cell surface analysis identified adhesive patches in rehydrated cell from strain L69

On the left panel (A) are shown AFM height (a, b), adhesion (a) and stiffness (d) images. The green hatched lines represent a cross-section drawn over a distance separated three identified patches. The change of height (in nm), adhesion (pN) and stiffness (pN/nm) is quantitatively represented by plotting the value of each of these biophysical parameter over the distance of this cross-section. The right panel (B) illustrates an AFM adhesion image at high resolution (z-scale: 2 nN) and force-distance curves obtained on a green, orange and red patches that are represented by the corresponding color in the bottom of this image.

Figure 4: Mapping mannan polysaccharides on the surface of a living and rehydrated cell from strain L71 using AFM tips functionalized with ConA

AFM height images (a, e), adhesion force maps (b, f), adhesion force histograms (n=1024) with representative force curves recorded with ConA tip (c, g) and plots of adhesion frequency versus

rupture distance (d, h) obtained on a living (a, b, c, d) and rehydrated sample (e, f, g, h) from strain L71. The living cell was from an exponential culture of L71 strain on YPD medium, whereas the rehydrated cell were from autolysed/dried sample of L71 strain obtained as "YCW" and rehydrated according to manufacturer's recommendation as described in Material & Methods.

Figure 5: Mapping mannan polysaccharides on the surface of a living and rehydrated cell from strain L69 using a AFM tip functionalized with ConA

AFM height images (a, c), adhesion force maps (b, f), adhesion force histograms (n=1024) with representative force curves recorded with ConA tip (c, g) and plots of adhesion frequency versus rupture distance (d, h) obtained on a lived (a, b, c, d) and rehydrated cell (e, f, g, h) from strain L69. The lived cell was from an exponential culture of L69 strain on YPD medium, whereas rehydrated cells were from "YCW" of strain L69 rehydrated according to manufacturer's recommendation as described in Material & Methods.

8. Supplementary data:

Figure S1: AFM images on rehydrated cell reveal the presence of a hole

AFM height images (a, b) recorded with QI™ mode (JPK instruments) and (c, d) plots of height versus distance of a cross-section represented by white hatched bars for rehydrated cell of strain L71 (a, b) and strain L69 (c, d). The bud and the hole identified of the height image are represented by B and H, respectively.

Figure S2. Interaction of conA-tip with surface polysaccharides is abolished by excess of mannose

Adhesion force maps (1 µm x 1 µm) with corresponding adhesion force histograms (n=1024) recorded with a Con A tip on rehydrated cell of L71 (a, b) and L69 (c, d) strain in the absence (a, c) or in the presence of 100 mM mannose (b, d).

Table 1: Cell wall composition of industrial strain L71 and L69

cell wall polysaccharides	**Culture YPD**		**Cream**		**Autolysis/drying**	
	Strain L71	Strain L69	Strain L71	Strain L69	Strain L71	Strain L69
	% of cell wall dry weight					
β-(1,3)-glucan	30.5 ± 6.0	36.2 ± 5.5*	34 ± 2.3	33.7 ± 5.0	32 ± 3.4	33.3 ± 4.0
β-(1,6)-glucan	24.4 ± 5.4	16.7 ± 1.7**	22.5 ± 5.5	17.3 ± 3.6**	22.5 ± 5.5	15.1 ± 2.7
chitin	6.0 ± 0.5	3.5 ± 1.0**	5.5 ± 1.0	2.8 ± 0.4**	5.5 ± 2.0	2.0 ± 0.4***
mannans	39.2 ± 7.0	43.5 ± 5.0**	37.0 ± 2.0	45.7 ± 5.5**	40.0 ± 2.0	48.5 ± 6.4**

The yeast strains L69 and L71 (Lallemand SAS collection) were cultivated in shake in flask under laboratory condition (Yeast Peptone Dextrose medium, 30°C) or obtained as 'Cream' or 'YCW' (autolysis/drying process (24 h at 55°C). The amounts of chitin, β-1,3-glucan, β-1,6-glucan and mannans in the cell wall are mean values ± SD obtained from three independent biological replicates, each made 2 times. Statistical comparisons in the cell wall composition between strains for each condition were made by one-way analysis of variance followed the Tukey's comparison test (XLstat software), for which *p* values were obtained indicated by an asterisk. *is meaning *p*-value < 0.05, ***p*-value <0.01 and ****p*-value <0.001.

Table 2: Effect of autolysis/drying process on cell surface roughness

Condition /treatment	Roughness (nm)	
	Strain L71	Strain L69
Culture YPD*	1.9 ± 0.2	1.7 ± 0.2
autolysis/drying	5.1 ± 1.1	6.4 ± 1.4

The yeast strain L69 and L71 (Lallemand Inc collection) were cultivated in shake in flask under laboratory condition (Yeast Peptone Dextrose medium, 30°C) or obtained as 'cream' or 'YCW' (autolysis/drying process (24h at 55°C). Surface roughness was calculated on AFM high resolution images of 1 x 1 µm on a total of 15 cells taken from three independent biological replicates for each condition.

Table 3: Difference in expression levels of genes related to cell wall biogenesis between L69 versus L71 strain

ORF	Gene	Biological function	Fold change
YER011W	*TIR1*	Cell wall mannoprotein	1.9
YGR166W	*KRE11*	β-1,6-glucan biosynthesis	2.0
YHR213W	*YHR213W*	Pseudogenic fragment, similar to flocculin	7.7
YIR019C	*FLO11*	flocculin, GPI-anchored cell surface glycoprotein	11.4
YJR151C	*DAN4*	Cell wall mannoprotein, similar to TIR1, TIR2, TIR3, and TIR4	1.7
YLR037C	*PAU23*	Cell wall mannoprotein, similar to TIR1, TIR2, TIR3, and TIR4	1.5
YLR194C	*YLR194C*	Unknown function and unknown phenotype	1.8
YOR382W	*FIT2*	GPI-anchored cell wall mannoprotein	3.1
YOR383C	*FIT3*	GPI-anchored cell wall mannoprotein	4.1
YER096W	*SHC1*	Sporulation-specific activator of Chs3p (chitin synthase III)	*0.5*
YGR279C	*SCW4*	Soluble cell wall protein with similarity to β-glucanases	*0.6*
YHR211W	*FLO5*	flocculin, lectin-like cell wall protein	*0.5*
YKL163W	*PIR3*	Member of PIR family protein, cell wall structural protein	*0.5*
YLR300W	*EXG1*	Exo-β-1,3-glucanase	*0.4*
YOR010C	*TIR2*	Putative cell wall mannoprotein	*0.5*
YOR140W	*SFL1*	Suppressor of flocculation	*0.7*

Table S1: Classification of genes whose expression was higher in strains L69 compared to L71 into MIPS categories*

Upregulated genes classified according to MIPS Functional Classification (459 categories)							
MIPS category	**Category**	**up-regulated genes in the given category**	**number of genes in MIPS category**	**% genes in each category**	**% genes in this category / total of upregulated genes**	**p-value**	**In category from Cluster**
transport facilitation	siderophore-iron transport	20	1038	1.9	11.4	8E-07	ARN1 ARN2 FET3 FRE4 ENB1 FRE3
	drug/toxin transport					3E-06	FLR1 AZR1 ARN1 ARN2 PDR11 QDR1 ENB1 YRM1 PDR10
	ABC transporters					2E-03	ARB1 PDR11 ENB1 PDR10 PXA1
metabolism & energy	fermentation	41	1881	2.2	23.0	7E-06	ADH7 AAD3 AAD4 AAD6 ALD2 AAD15 ATF1
	tetracyclic and pentacyclic triterpenes (cholesterin, steroids and hopanoids) metabolism					1E-05	ERG28 ERG25 ERG11 ERG7 ERG3 ERG27 ERG6 HMG1
	C-compound and carbohydrate metabolism					3E-04	GPI18 ADH7 AAD3 AAD4 YEL047C AAD6 ATF2 PHO12 FLO11 KTI12 GAL80 ALD2 PGM3 YNR071C YNR073C CSI2 AAD15 ATF1
	metabolism of phenylalanine					6E-04	AAD3 AAD4 AAD6 AAD15
	metabolism of tyrosine					8E-04	AAD3 AAD4 AAD6 AAD15
stress response	detoxification by export	9	554	1.6	5.0	3E-03	QDR1 YRM1 FLR1 AZR1 ARN1 ARN2 YLR046C ENB1 SSU1
unclassified proteins	unclassified proteins	60	1393	4.3	34.0	6E-03	PAU8 YAR064W YBL044W PSY4 YBL055C APD1 YBR242W HBN1 RNQ1 YCR051W YDR056C RTN1 FDC1 YEL057C YER077C AIM11 YER137C YFL051C PAU11 YGR017W ECL1 HGH1 PAU12 YHL042W YHR078W YHR213W YIL086C YIL096C YJL213W YJR039W YJR056C YJR115W DAN4 YKL047W COS9 YLL056C PAU18 PAU23 AVL9 YLR194C YLR326W YLR346C YMR007W YMR178W YMR181C YMR265C RSN1 YNL022C TCB2 YNL095C YNL193W YOL166C YOR012W IRC11 YOR342C YOR385W YOR390W YPL068C YPL279C YPR071W
regulation of metabolism and protein function	protease inhibitor	2	253	0.8	1.1	9E-03	RAD23 TFS1
others	in several non-enriched categories	43			25.0		

* Differential genes expression between L69 and L71 strain were retained on the basis of fold change > 1.5 at *p*-value <0.01

Table S2: Classification of genes whose expression was lower in strains L69 compared to L71 into MIPS categories*

Downregulated genes classified according to MIPS Functional Classification (459 categories)							
MIPS category	**Category**	**down-regulated genes in the given category**	**number of genes in MIPS category**	**% genes in each category**	**% of genes in this category / total of downregulated genes**	**p-value**	**In category from Cluster**
cell cycle and DNA progressing	DNA topology	14	1012	1.4	6.5	7E-06	YBL113C YEL077C YRF1-2 YRF1-3 YIL177C YJL225C YLL067C YRF1-6 YRF1-7 YPR204W
	somatic / mitotic recombination					4E-03	YRF1-2 YRF1-3 YRF1-6 YRF1-7
communication	G-protein mediated signal transduction	6	234	2.6	2.8	5E-03	GPB2 STE18 STE4
	cAMP/cGMP mediated signal transduction					6E-03	PDE1 RAS1 SFL1
metabolism	sulfate assimilation	42	1514	2.8	19.4	3E-10	MET10 MET3 MET5 MET14 MET1 MET22 MET16
	purine nucleotide/nucleoside/nucleobase anabolism					2E-07	ADE1 ADE5,7 ADE3 MTD1 ADE13 IMD3 ADE4 ADE2 SER1
	biosynthesis of methionine					2E-05	CBF1 MET14 MET2 MET22
	metabolism of methionine					5E-04	MET32 MET3 MET1 MET17 MET16
	degradation of glycine					6E-04	GCV3 GCV1 SHM2
	biosynthesis of serine					1E-03	SER33 SHM2 SER1
	biosynthesis of homocysteine					1E-03	MET10 STR3 MET5
	biosynthesis of leucine					2E-03	LEU2 LEU1 BAT1
	tetrahydrofolate-dependent C-1-transfer					8E-03	ADE3 MTD1 SHM2
	C-1 compound catabolism					1E-02	GCV3 GCV1
protein binding	NAD/NADP binding	7	1049	0.7	3.2	1E-04	GPD1 MET10 ADE3 SER33 MET5 MTD1 GPD2
transport facilitation	sulfate/sulfite transport	2	1038	0.2	0.9	3E-03	OAC1, SUL2
others	in several non-enriched categories	143			67.3		

*Differential genes expression between L69 and L71 strain were retained on the basis of fold change < 0.6 at *p*-value <0.01