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1 Effects of the strain background and autolysis process on the composition and biophysical properties
2 of the cell wall from two different industrial yeasts

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16

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

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

19

20 **Abstract**

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

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41

42 1. Introduction

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

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

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

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

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

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

122 2. **Material** and Methods

123 2.1. **Strains and culture conditions**

124 Two diploid industrial winemaking *Saccharomyces cerevisiae* strains, L71 and L69, from
125 Lallemand Inc. (Blagnac, France) were studied. Differences between the two strain was mainly that
126 the L69 strain has a higher mannoproteins content, expresses different sensorial notes during wine
127 fermentation and presents good capacity to interact with volatile undesirable phenols (Lallemand
128 Inc., unpublished data). The strains were propagated under standard laboratory conditions, which
129 corresponded to growth in 200 ml YPD medium (1% [w/v] yeast extract, 2% [w/v] bactopectone and
130 2% [w/v] glucose) in 1 liter shake flasks at 30°C under shaking at 200 rpm. They were also propagated
131 under industrial standard protocol, involving batch and fed-batch growth on molasses based
132 medium, collected, and concentrated to produce packed yeast cells termed 'cream'. The latter
133 cream was then subjected to autolysis (20 **hr** at 55°C), followed by separation and then by spray-
134 drying to produce the dry "YCW" (industrial term meaning Yeast Cell Wall fraction that is used for
135 this type of yeast packaging). This autolytic/drying process leads to > 99.9 % of cell mortality as
136 estimated by methylene blue coloration test {Cot, 2007 5468 /id}. YCW were provided in 30 g packets
137 sealed under vacuum and stored unopened at 4°C until required. Rehydration was performed
138 according to the manufacturer's guidelines (Lallemand Inc., Blagnac, France): 0.1 g of YCW was
139 sprinkled onto 10× its weight of temperature equilibrated (30°C) sterile water in a tube. The YCW
140 was left to absorb water slowly for 20 min, mixed gently to form slurry and the temperature was
141 maintained at 30°C throughout rehydration.

142 2.2. **AFM measurements**

143 2.2.1. *Sample preparation*

144 Cells collected from exponential growth in YPD or after rehydration of YCW were washed two
145 times in sodium acetate (18 mM CH₃COONa, 1 mM CaCl₂ and, 1 mM MnCl₂, pH 5.2), and immobilized
146 on polydimethylsiloxane (PDMS) stamps prepared as described in {Dague, 2011 442 /id}. Briefly,
147 freshly oxygen activated microstructured PDMS stamps were covered by 100 µL of yeasts sample at

148 OD₆₀₀ 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 {Chopinnet, 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}:

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

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

174 $\mu\text{g}\cdot\text{ml}^{-1}$ in a 0.1 M carbonate buffer). After 1 hr incubation, 100 μL of NaBH_4 $3.5\text{ mg}\cdot\text{mL}^{-1}$ solution was
175 added and incubated 15 min in order to reduce the unreacted groups. Finally, the cantilever bearing
176 the functionalized tip was washed three times and stored in acetate buffer. To analyze the stretching
177 of polysaccharides at the surface of the cell, elongation forces were stretched using the worm-like
178 chain (WLC) model introduced by Bustamante {Bustamante, 1994 5811 /id} which describes the
179 polymer as a curved filament and the force F vs the extension x is given by:

$$180 \quad F(x) = k_b T / l_p [0.25 (1-x/L_c)^{-2} + x/L_c - 0.25]$$

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

187 **2.3. Cell wall isolation and quantification of polysaccharides**

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

196 **2.4. Transcriptomic analyses**

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

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

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

225

226

227 **3. Results**

228 **3.1. Effects of autolysis/drying process on cell wall composition of two industrial yeast strains.**

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

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

254 **3.2. Effects of autolysis/drying process on nanomechanical properties of two industrial yeast** 255 **strains.**

256 In accordance with a previous work {Hernawan, 1995 4965 /id}, rehydrated YCW regained
257 round to ovoid cell shape like a normal living yeast cell (data not shown). This cell shape retention
258 allowed using our immobilization methodology that is based on trapping of single round/ovoid yeast
259 cell into microchambers made in microstructured PDMS stamp by capillary/convective means
260 {Dague, 2011 442 /id}. This methodology is very efficient to readily monitor several isolated cells in
261 liquid environment by AFM. Topographic images using Quantitative Imaging™ mode {Chopinot, 2013
262 454 /id} of living yeast cells from strain L71 and rehydrated YCW derived from L71 strain is reported
263 in Figure 1 (a and b). For both types of yeast samples, height images illustrated a rather round shape
264 of the immobilized object with an apparent smooth surface. However, a closer inspection of the AFM
265 images from the rehydrated sample revealed the presence of a single hole of 600 nm diameter size
266 curiously localized in the middle of immobilized cell (Fig. S1 in supplementary data), and this feature
267 was obtained in at least 15 % of the analyzed cells. At higher resolution, height images (Fig.1 c and d)
268 from contact mode clearly showed significant differences of the surface between living and
269 rehydrated sample from L71 strain, as the surface of the latter was severely fractured. In quantitative
270 terms, the roughness of cell surface (determined on 1 μm x 1 μm areas) was around 1.9 ± 0.2 nm for
271 the living cell and increased to 5.1 ± 1.1 nm for the rehydrated YCW sample (Table 2). This surface
272 change could be linked to the ultrastructural changes that was reported by Low Temperature
273 Scanning Electron Microscopy (LSTEM) of a commercial yeast strain after 14 hr of induced autolysis in
274 a model wine medium {Martinez-Rodriguez, 2001 4964 /id}. Figure 1 (e, f) shows adhesion images of
275 a living and rehydrated cell from YCW of L71 strain that were obtained by probing the surface with
276 an AFM tip in QI™ mode. As the tip is made of silicon nitride and the immobilized sample is under an
277 acidic pH (sodium acetate 18 mM at pH 5.2), these conditions most likely favor hydrophobic

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

280 Similar AFM analyses were carried out with active living yeast cells and rehydrated YCW of
281 L69 strain. The topographic images taken at low resolution (Fig 2, a and c) did not show difference in
282 both types of cell samples immobilized in PDMS (Fig. 2). At higher resolution, some discretional dots
283 could be visualized that were rather larger in rehydrated YCW (Fig 2, b and d). In addition, we found a
284 hole in immobilized rehydrated samples of YCW in about at least 15 % of the AFM images, like it was
285 seen for strain L71 (see Fig.S1 supplementary data). The force curves acquired in QI mode were also
286 analyzed to obtain adhesion images. This analysis revealed adhesives patches that were not present
287 in L71 strain and which became apparently bigger in the sample from rehydrated YCW (compared
288 Fig. 2 a, e, i for active yeast cells with c, g and k for rehydrated YCW). On average, the patches from
289 the rehydrated YCW had a mean area of $156663.4 \pm 3904.4 \text{ nm}^2$ ($0.016 \pm 0.004 \text{ }\mu\text{m}^2$) and a diameter
290 of $141 \pm 32 \text{ nm}$ (measured from 56 patches on 7 different cells with Image J software). Additional
291 details of the morphology and mechanical properties of these patches are reported in Figure 3 and
292 Figure S2 in supplementary data. From a cross-section taken through several patches (see the green
293 dashed line), the corresponding height, adhesion and stiffness values were determined. This detailed
294 AFM analyses showed that these patches corresponded to small protuberances exceeding the cell
295 surface as shown in Figure 3Ae (also shown in Figure S2 b and d, in supplementary data) by about 30
296 ~~40 nm~~ (Fig. 3A). These protuberances strongly interacted with the AFM tip (Fig. 3B). Example of
297 adhesion force is schematically illustrated by force-distance curves taken from 3 different patches
298 (Fig. 3B). On the contrary, these patches exhibited lower stiffness than the ground surface of the
299 rehydrated YCW sample. Recently, similar observation was made with other *Candida albicans* cells
300 that also harbored nanodomains at its cell surface represented by hydrophobic patches that were
301 likely due to high expression the *ALS1* encoding cell surface protein (Alsteens et al., 2010; Formosa et
302 al., 2014, Nanomedicine). Taken together, these results provide some clues about the surface
303 properties of L69 strain which were distinct from those of L71 strain.

304

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

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

365 **4. Discussion**

366 Combination of biochemical analyses and AFM technology allows us to observe that
367 packaging yeast cells as “YCW” by autolysis and drying process significantly affected the global
368 surface properties of the cells, in spite of the fact that the biochemical composition of the cell wall
369 was barely altered by this packaging process. This result reinforces our previous finding showing no
370 direct relationship between the polysaccharides composition of cell wall and its bionanomechanical
371 properties {Dague, 2010 5052 /id;Pillet, 2014 5726 /id;Formosa, 2013 5624 /id}. Also, the finding that
372 cell wall composition remained barely unchanged after autolysis and drying process is at variance to
373 other reports that indicated some loss of β -glucan and mannans during this process {Cavagna, 2010
374 4950 /id;Giovani, 2007 4954 /id}. However, the discrepancy can be explained by the difference in the
375 autolysis process. In our study, it is an industrial process that consisted in an induced autolysis at high
376 temperature for 20 h, whereas other studies were related to yeast autolysis during wine
377 fermentation that lasted several days and was done at lower temperature {Martinez-Rodriguez, 2009
378 455 /id;Alexandre, 2006 456 /id}. As compared to living or active yeast cells, the main effect of
379 autolysis/drying process was to increase the roughness of the cell surface, which was visualized as a
380 change from a relatively smooth to wrinkled structure. It remains to evaluate whether this
381 modification has some impact in the sorption capacity of the yeast cells.

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

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

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

421

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428

429 **6. References**

430

431

432 **7. Legend to figures:**

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

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

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

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

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

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

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

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

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

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

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

468

469 **8. Supplementary data:**

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

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

475

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

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

480

481

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 \pm 6.0	36.2 \pm 5.5*	34 \pm 2.3	33.7 \pm 5.0	32 \pm 3.4	33.3 \pm 4.0
β -(1,6)-glucan	24.4 \pm 5.4	16.7 \pm 1.7**	22.5 \pm 5.5	17.3 \pm 3.6**	22.5 \pm 5.5	15.1 \pm 2.7
chitin	6.0 \pm 0.5	3.5 \pm 1.0**	5.5 \pm 1.0	2.8 \pm 0.4**	5.5 \pm 2.0	2.0 \pm 0.4***
mannans	39.2 \pm 7.0	43.5 \pm 5.0**	37.0 \pm 2.0	45.7 \pm 5.5**	40.0 \pm 2.0	48.5 \pm 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 \pm 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	<i>TIR1</i>	Cell wall mannoprotein	1.9
YGR166W	<i>KRE11</i>	β -1,6-glucan biosynthesis	2.0
YHR213W	<i>YHR213W</i>	Pseudogenic fragment, similar to flocculin	7.7
YIR019C	<i>FLO11</i>	flocculin, GPI-anchored cell surface glycoprotein	11.4
YJR151C	<i>DAN4</i>	Cell wall mannoprotein, similar to TIR1, TIR2, TIR3, and TIR4	1.7
YLR037C	<i>PAU23</i>	Cell wall mannoprotein, similar to TIR1, TIR2, TIR3, and TIR4	1.5
YLR194C	<i>YLR194C</i>	Unknown function and unknown phenotype	1.8
YOR382W	<i>FIT2</i>	GPI-anchored cell wall mannoprotein	3.1
YOR383C	<i>FIT3</i>	GPI-anchored cell wall mannoprotein	4.1
YER096W	<i>SHC1</i>	Sporulation-specific activator of Chs3p (chitin synthase III)	0.5
YGR279C	<i>SCW4</i>	Soluble cell wall protein with similarity to β -glucanases	0.6
YHR211W	<i>FLO5</i>	flocculin, lectin-like cell wall protein	0.5
YKL163W	<i>PIR3</i>	Member of PIR family protein, cell wall structural protein	0.5
YLR300W	<i>EXG1</i>	Exo- β -1,3-glucanase	0.4
YOR010C	<i>TIR2</i>	Putative cell wall mannoprotein	0.5
YOR140W	<i>SFL1</i>	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