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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

3

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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,  $\beta$ -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  $\beta$ -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  $\beta$ -(1,3) glucose  
61 residues are branched to  $\beta$ -(1,6)-glucans, forming a fibrillar network, that serves as backbone to  
62 which are linked chitin,  $\beta$ -(1,6)-glucan and mannoproteins. In addition, the outer layer of  
63 mannoproteins can be linked to the inner layer through  $\beta$ -(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  $\beta$ -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,  $\beta$ -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  $\beta$ -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 CH<sub>3</sub>COONa, 1 mM CaCl<sub>2</sub> and, 1 mM MnCl<sub>2</sub>, 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 OD<sub>600</sub> 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<sup>-1</sup>. 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<sub>cell</sub> in N.m<sup>-1</sup>) 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  $\mu\text{L}$  of  $\text{NaBH}_4$   $3.5\text{ mg}\cdot\text{mL}^{-1}$  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,  $\beta$ -(1,3) and  
191  $\beta$ -(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  $\text{OD}_{600}$  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-  
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](http://www.R-project.org)) using  
211 the Limma package {Yang, 2002 2877 /id} ([www.bioconductor.org](http://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  $\beta$ -(1,3),  $\beta$ -(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  $\beta$ -(1,6)-glucan in total  $\beta$ -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  $\mu\text{m}$  x 1  $\mu\text{m}$  areas) was around  $1.9 \pm 0.2$  nm for  
271 the living cell and increased to  $5.1 \pm 1.1$  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  $\alpha$ -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 ( $58 \pm 21$   
319 pN for the living cell and  $65 \pm 38$  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  $\beta$ -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  $\beta$ -branched amino acids (Ile, Thre, Val) that are predicted to form  
395 intramolecular  $\beta$ -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  $\beta$ -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  $\beta$ -(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  $\beta$ -(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  $\beta$ -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  $\mu\text{m}$  x 1  $\mu\text{m}$ ) 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						
$\beta$ -(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
$\beta$ -(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,  $\beta$ -1,3-glucan,  $\beta$ -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>	$\beta$ -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 $\beta$ -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- $\beta$ -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