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Effects of Yeast Cell-Wall Characteristics on 4-Ethylphenol Sorption Capacity in Model Wine

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Saccharomyces cerevisiae is an efficient biosorbant, used in winemaking to reduce the concentration of undesirable molecules such as fatty acids. Volatile phenols such as 4-ethylphenol, which causes a horsy smell in wine, are particular targets of this type of curative process. This study demonstrates that the sorption capacity of 4-ethylphenol by yeasts is greatly influenced by strain nature, methods, and medium used for biomass production and drying after harvesting. *S. cerevisiae* mutant strains with deletion of genes encoding specific proteins involved in cell-wall structure and composition were studied, and a major role for mannoproteins in 4-ethylphenol sorption was identified. It was confirmed that 4-ethylphenol sorption occurs at the surface of the yeast wall and that not all mannoproteins are determinants of sorption: the sorption capacity of cells with deletion of the Gas1p-encoding gene was 75% lower than that of wild type. Physicochemical properties of yeast cell surface have been also studied.

KEYWORDS: Yeast cell wall; mannoproteins; sorption; 4-ethylphenol

INTRODUCTION

Contamination by the yeast *Brettanomyces bruxellensis* during the winemaking process can have major consequences on the organoleptic profile of wine (*1*). Depending on growth conditions, this microorganism can produce volatile phenols such as 4-ethylgaiacol, 4-ethylcatechol, and 4-ethylphenol (*1*). A concentration of 4-ethylphenol of $\geq$440 μg L^{-1} in red wine can cause an unpleasant aroma, described as "horse sweat", "medicinal", "stable", and "spicy". The growth of *Brettanomyces* in wine therefore needs to be prevented. Biological methods for preventing contamination by yeasts involve the use of fining agents, filtration, antimicrobial agents, and high pressure (*2*). However, *Brettanomyces* contamination is still widespread. Curative treatment is thus required. Ethylphenol concentration can be reduced by reverse osmosis (*3*), PVPP, or charcoal (*2*). The main disadvantage of these methods is a reduction in aromatic compound concentration and color in red wines. The use of yeast hulls represents an ecofriendly alternative to conventional physicochemical techniques used to decrease the volatile phenol content in wine. Indeed, yeast hulls have been used in wine as a biosorbent of undesirable molecules such as octanoic and decanoic acids (*4*). Previous studies have demonstrated the sorption capacity of yeasts for different wine components, including phenolic compounds (*5*), sulfur products (*6*), and aroma compounds (*7*) such as volatile phenols. Guilloux-Benatier et al. (*8*) compared the same wine aged with and without lees. They found that 4-ethylphenol concentration was lower in the presence of lees (*8*). This observation could be explained by sorption of the volatile phenol by yeasts (*9*).

Sites of interaction between molecules and yeasts should be localized on the yeast wall; however, the nature of these interactions has been poorly studied, and contradictory findings have been reported.

It appears that the physicochemical characteristics of the molecule are important factors determining the level of sorption. Using model media, some studies conclude that the rate of sorption by yeasts is directly correlated with the hydrophobicity of the aroma compound (*7*). However, Jiménez Moreno et al. (*10*) showed that lees had a considerable sorption capacity for volatile phenols with low hydrophobicity, such as eugenol, 4-propylguaiacol, or 4-methylguaiacol, in real wine. Mazauric and Salmon (*11*) also disagree about the influence of the hydrophobicity. According to these authors, the sorption of phenolic compounds on yeast wall is unrelated to the polarity. However, although the nature of these compounds was more or less hydrophobic, the characteristics of the yeast cell surface are unknown. These previous findings suggest that weak interactions, such as hydrophobic, electrostatic, and van der Waals forces, could be involved in molecular interactions with the yeast wall. Thus, the study of yeast wall physicochemical

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Table 1. Characteristics of Yeast Mutant Strains

strain (Orf)	deleted gene	characteristics of the encoded protein	characteristics of the mutant strain	refs
YIR019c	*muc1*	hydrophobic cell-wall mannoprotein with GPi anchor, involved in velum formation, adhesion properties	*a*	*20, 32*
YKL096w	*cwp1*	cell-wall mannoprotein with GPi anchor	*a*	*33*
YBR067c	*tip1*	major cell-wall mannoprotein, possible lipase activity with GPi anchor	*a*	*33*
YGR229c	*smi1*	protein involved in the regulation of synchronization of wall biosynthesis with cellular cycle	increased wall chitin levels, high amount of mannoproteins released in the medium, lower wall glucan content, decreased resistance to calcofluor white	*21, 34*
YLR342w	*fks1*	catalytic subunit of 1,3-D-glucan	increased wall chitin levels, decreased resistance to calcofluor white, growth defect on a fermentable carbon source	*35*
YMR307w	*gas1*	β-1,3-glucanosyltransferase with GPi anchor linked to the plasma membrane	increased wall chitin levels and mannoproteins, large amounts of mannoproteins released in the medium, decreased resistance to calcofluor white	*36, 37*
YJL062w	*las21*	integral plasma membrane protein involved in the GPi anchor synthesis	increased wall chitin levels, large amounts of mannoproteins released in the medium, decreased resistance to calcofluor white	*24, 38*
YFL014w	*hsp12*	plasma membrane protein, protects membrane from desiccation, involved in velum formation	wall permeability decreased	*39, 40*

[a] No information available.

properties could provide insight into the nature of such interactions, and useful predictive tools could be developed if these properties are found to be correlated with sorption rates. The characterization of the physicochemical properties of a microorganism's surface is a common approach in the study of cell−material interaction (*12*) to determine the nature of the interactions involved. Surface properties of yeast cells also depend on the wall biochemical composition. Growth conditions, such as culture method and nutrient availability (*13*), affect cell-wall composition. Consequently, cell-wall composition varies between cells of the same strain cultivated with different growth parameters. Thus, the surface physicochemical properties and sorption capacity of yeast may depend on yeast strain and culture conditions.

The yeast wall is a complex dynamic structure with a composition dependent on a variety of factors. Wall composition is directly determined by the strain genome, but changes occur during the cell cycle (*14*).

The yeast wall is composed of a three-dimensional internal skeletal layer of 1,3-β glucan (30−45% of wall mass) stabilized by hydrogen bonds. Mannoproteins (30−40% of wall mass), or cell-wall proteins, are linked to the nonreducing ends of 1,3-β-glucan (Pir-CWPs) or 1,6-β-glucan molecules (5−10% of wall mass) (GPI-CWPs). Mannoproteins are the most highly exposed cell-wall molecules and may therefore form sorption sites.

The main purpose of this study was to characterize the sorption capacity of 4-ethylphenol in a model wine of six *Saccharomyces cerevisiae* (*S1*, *S2*, *S3*, *S4*, *S6*, and *S7*) strains and one strain of *Saccharomyces uvarum* (*S5)* grown under three different culture conditions, in order to determine the main factors involved (conditions of culture, yeast strain, and cell-wall characteristics). We determined the physicochemical surface properties of each cell population—surface hydrophobicity, charge, and electron donor characteristics and the specific area of cell contact during experiments—and their effects on sorption capacity of the different strains.

To identify cell-wall compounds involved in the sorption mechanism, we studied the effects of deletions of various genes encoding mannoproteins or genes involved in yeast wall metabolism on the sorption of 4-ethylphenol in yeast.

MATERIALS AND METHODS

Chemicals. The following chemicals were used: glucose, fructose, peptone, tartaric acid, yeast nitrogen base without amino acids, malic acid, ammonium chloride, potassium hydroxide (99%), potassium sulfate (99%), sodium chloride (99%), 4-ethylphenol (99%), 3,4-dimethylphenol (99%), acetic acid (98%), magnesium sulfate (98%), malic acid (99%), decane (99%), hexadecane (99%), absolute ethanol (99.8%), chloroform (99%), and ethyl acetate (99.5%). These chemicals were obtained from Sigma (St. Louis, MO). Solutions were made up with ultrapure water, obtained from a Milli-Q system (Millipore, Bedford, MA).

Yeasts and Culture Medium. We used seven enological yeast strains to study the effect of strain on 4-ethylphenol sorption capacity by yeast. Each of these strains has specific characteristics: *S1* is an acid must-adapted strain; *S2* has high protease activity; *S3* is a classical enological strain; *S4* has a long maceration period; *S5* is a *S. uvarum* strain; *S6* forms velum; and *S7* has a high alcohol tolerance. Enological strains and eight laboratory mutant strains (**Table 1**) (*YIR019C*, *YBR067c*, *YKL096w*, *YMR307w*, *YGR229c*, *YJL062w*, *YLR342w*, and *YFLO14w*) (Euroscarf, Frankfurt, Germany) and the wild type (*BY4741*) strain were cultivated in YPD medium (yeast extract, 5 g L^{-1}; peptone, 10 g L^{-1}; and D-glucose, 20 g L^{-1}) at 30 °C in dynamic mode (80 rpm) under aerobic conditions to obtain a sufficient quantity of biomass. Enological strains were also cultivated in synthetic must [glucose, 75 g L^{-1}; fructose, 85 g L^{-1}; tartaric acid, 2 g L^{-1}; malic acid, 10 g L^{-1}; ammonium chloride, 1.5 g L^{-1}; and 6.7 g L^{-1} of yeast nitrogen base without amino acids; pH 3.5 with potassium hydroxide solution (5 M)] at 20 °C in static mode.

Yeast cells were harvested in the early stationary phase of growth for strains in YPD culture and after alcoholic fermentation for strains grown in synthetic must. Cells were then washed three times with sterilized distilled water.

We also carried out these tests for the active dry yeast (ADY) form of three enological strains (*S1*, *S2*, and *S3*). ADY cells were used immediately after hydration (20 min in sterilized deionized water with 5% m/m glucose at 30 °C) followed by three washes with sterilized distilled water.

Model Wine. The model wine buffer contained ethanol (12.5%, v/v), D,L-malic acid (3 g L^{-1}), acetic acid (0.1 g L^{-1}), potassium sulfate (0.1 g L^{-1}), and magnesium sulfate (0.025 g L^{-1}). The pH was adjusted to 3.5 with potassium hydroxide (2 N).

Sorption Measurements. Sorption capacity was determined in model wine with yeast biomass (50 g of fresh biomass L^{-1}) stored in 37 mL capacity flasks, with Teflon caps to prevent loss of volatile compounds. The initial concentration of 4-ethylphenol in model wine was 10 ppm. Samples were stirred (550 rpm) at 10 °C until equilibrium was reached. The pH (3.5), ethanol content (12.5%) of the synthetic wine, and temperature of the studied system (10 °C) were fixed because these parameters have a significant effect on 4-ethylphenol sorption by yeast (*9*). The amount of volatile phenols sorbed was calculated from the difference in concentration between control samples (sample without biomass) and test samples. For all modalities studied, this test on 4-ethylphenol sorption was made in triplicate.

Volatile Phenol Analysis. Samples (2 mL) were centrifuged (5000*g* at 4 °C for 5 min) to remove yeast. For gas chromatography analysis, 10 μg of 3,4-dimethylphenol (10 μg; internal standard) was added to 1 mL of the supernatant in a 4 mL vial with a Teflon cap. We used a Focus GC apparatus (Thermo-Finnigan) equipped with a capillary column CP Wax 57 CB (25 m × 0.25 mm i.d., 0.2 μm bonded phase) (Varian) and FID detector. The column temperature was programmed to increase at 3 °C min^{-1} from 170 to 190 °C. Temperatures for the injector and detector were kept at 200 and 250 °C, respectively. The flow rate of the carrier gas (He) was 1.1 mL min^{-1}, 30 mL min^{-1} for H_2, 350 mL min^{-1} for air, and 35 mL min^{-1} for N_2 makeup. Data acquisition and treatment were carried out using Chrom-card workstation version (Thermo Electron Corp.).

Physicochemical Properties of Yeast Cell Surface. Cells were harvested in the early stationary phase of growth by centrifugation (5000*g*, 4 °C, 10 min) and washed twice with deionized sterilized water. All tests were performed in triplicate.

Electron Donor/Acceptor Properties of Surface Cells. The microbial adhesion to solvent (MATS) technique was used to determine the electron donor or acceptor properties of yeast cells (*15*). MATS is based on cell surface affinities for monopolar and nonpolar solvents. We used chloroform and hexadecane because they have the same Lifshitz−van der Waals component of their surface tension. The acidic nature of chloroform allows the electron donor capacity to be determined.

We added 0.4 mL of solvent to 2.4 mL of yeast suspension in physiological water [pH adjusted to 3.5 with hydrogen chloride (2 N), 9 g of NaCl L^{-1} of sterilized distilled water] in a glass tube. The yeast suspension had an initial absorbance of 0.7 ($Abs_{600initial}$). The mixture was stirred (1 min) and subjected to phase separation (15 min) before final absorbance at 600 nm of the aqueous phase was measured ($Abs_{600final}$).

The proportion of cells bound to each solvent was calculated as affinity = $(1 - Abs_{600final}/Abs_{600initial}) \times 100$, and the electron donor character was calculated as electron donor character = $(affinity_{chloroform} - affinity_{hexadecane})$.

Cell Surface Hydrophobicity. Yeast cell surface hydrophobicity was determined by adhesion of yeast cells to paramagnetic, polystyrene-coated latex beads (*16*). We added 15 μL of the beads (1−2 μm; Polysciences, Inc., Warrington, PA) to 985 μL of yeast suspension in 50 mM sodium acetate buffer (pH 4.5) in a glass tube. The yeast suspension had an initial absorbance of 0.4 ($Abs_{660initial}$). After continuous shaking (20 min, 30 °C, 80 rpm), the tube was placed against a neodymium iron magnet (Polysciences, Inc.) for 2 min, and the absorbance at 660 nm of the suspension was measured ($Abs_{660final}$). The percentage of cells adhering to the beads was calculated as $\%A = [(Abs_{initial} - Abs_{final})/Abs_{initial}] \times 100$.

Zeta Potential of Cell Surfaces. Yeast surface charge was quantified by measurement of the electrophoretic mobility of cells on laser zetacompact (CAD Instrumentation, France). This allows calculation of the zeta potential (mV) using Smoluckowsky's equation. An electric field of 8 V cm^{-1} was applied to a yeast suspension of 2×10^6 cells mL^{-1} in sterile physiological water [pH adjusted to 3.5 with hydrogen chloride (2 N)].

Table 2. Mean Diameters of the Enological Strains as a Function of Biomass Production Method[a]

	strain	mean diameter of cells (μm)
ADY	*S1*	3.90 b ± 0.02
	S2	3.84 c ± 0.04
	S3	3.97 a ± 0.00
AF	*S1*	4.95 g ± 0.02
	S2	4.95 g ± 0.03
	S3	4.99 gh ± 0.02
	S4	5.02 h ± 0.02
	S5	5.45 j ± 0.03
	S6	5.15 l ± 0.01
	S7	4.66 f ± 0.02
YPD	*S1*	4.55 e ± 0.00
	S2	5.40 j ± 0.02
	S3	6.06 l ± 0.00
	S4	6.05 l ± 0.02
	S5	4.18 d ± 0.00
	S6	5.53 k ± 0.01
	S7	4.66 f ± 0.02

[a] ADY, biomass obtained after hydration of the active dry yeast; AF, biomass obtained after alcoholic fermentation in synthetic must; YPD, biomass obtained after culture in YPD medium. Different letters following diameters indicate significant difference indicated ($p < 0.05$) by ANOVA text.

Cell Size. Yeast cell size was determined using granulometry laser (Mastersizer Hydro 2000 SM, Malvern Instruments, U.K). Yeast cells were suspended in physiological water for measurements.

Statistical Analysis. Data are presented as the mean ± standard deviation (SD). Statistical comparisons were made by one-way analysis of variance followed by Tukey's comparison test (XLstat software). A p value of <0.05 was considered to be significant.

The modelization of the 4-ethylphenol sorption by yeasts according to the surface physicochemical properties parameters was performed using multiple linear regressions (XLstat software). This analysis by comparison of the sorption capacities measurement to the physicochemical parameters of yeast surface permit the construction of a mathematical model of prediction with three variables (surface hydrophobicity, electron donor character, and zeta potential). Coefficients are attributed to each physicochemical parameter of the yeast surface.

These coefficients allowed identifying significant or not (a p value of <0.05 was considered to be significant) parameters explaining the sorption phenomenon and the negative or positive influence of the parameters involved in 4-ethylphenol sorption.

RESULTS

In real enological conditions, organoleptic defects of wine caused by 4-ethylphenol can be detected at concentrations of ≥440 μg L^{-1}. The 4-ethylphenol concentration in wine can reach concentrations approaching milligram per liter. In our study, we used higher concentrations (10 ppm) to allow direct gas chromatography analysis of 4-ethylphenol and to minimize variability in measurements.

Given that the key step of 4-ethylphenol sorption by yeast is thought to be the binding of the volatile phenol to the cell surface (*9*), the sorption capacity of yeast was expressed as the quantity of 4-ethylphenol sorbed (mg) per cell surface area (m^2) during the course of the experiment. This expression of sorption in mg m^{-2} allows sorption to be normalized against the yeast surface. Indeed, significant differences in the mean size of cells have been observed between strains and between cell populations of the same strain cultivated in different media (**Table 2**). These differences in cell size can markedly affect the comparison

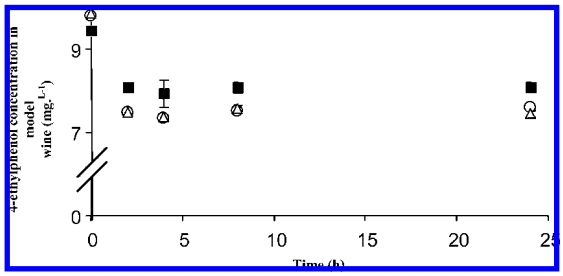

Figure 1. Changes in 4-ethylphenol concentration over time in a model wine according to a different kind of *S2* strain biomass (50 g L^{-1} of fresh biomass) at 10 °C: (○) from culture in YPD medium; (■) after alcoholic fermentation in synthetic must; (△) after hydration of the active dry yeast.

Table 3. Sorption Capacities of the Enological Strains as a Function of Biomass Production Method[a]

	4-ethylphenol sorption capacity (mg m^{-2})		
strain	ADY	AF	YPD
S1	0.882 c ± 0.003	0.527 fg ± 0.030	0.158 k ± 0.010
S2	0.819 d ± 0.019	0.676 e ± 0.013	0.316 j ± 0.031
S3	0.568 f ± 0.005	0.512 fg ± 0.020	0.322 ij ± 0.014
S4		1.697 a ± 0.027	0.433 h ± 0.017
S5		0.963 b ± 0.001	0.317 j ± 0.006
S6		0.702 e ± 0.026	0.373 l ± 0.001
S7		0.489 g ± 0.011	0.337 ij ± 0.011

[a] ADY, biomass obtained after hydration of the active dry yeast; AF, biomass obtained after alcoholic fermentation in synthetic must; YPD, biomass obtained after culture in YPD medium. Values followed by different letters are significantly different ($p < 0.05$) by the ANOVA test.

of 4-ethylphenol sorption capacity between samples. That is why the mass partition coefficient ($K_{mas} = C_{yeast}/C_{modelwine}$, where C_{yeast} and $C_{modelwine}$ are the volatile phenol concentrations expressed in mg kg^{-1} at the yeast surface and in the model wine, respectively, at equilibrium) or the sorption ratio, which do not take the cell surface area into account, were not used to compare sorption capacities.

The change over time of 4-ethylphenol concentration in model wine in the presence of yeast is illustrated in **Figure 1**. Equilibrium of the sorption kinetic was reached after 4 h of contact time. No further change in the equilibrium state was observed after 24 h of storage, confirming previous reports of the rapid sorption kinetics of 4-ethylphenol (*9*) and anthocyanins (*5*) by yeast lees. In this case, the 4-ethylphenol concentration in the model wine was decreased by 16% (calculated from the initial concentration in the model wine and the final 4-ethylphenol concentration at the equilibrium state) at equilibrium with the cells cultivated in YPD medium and decreased by 25% for the ADY form after alcoholic fermentation (**Figure 1**).

Due to the different physiological characteristics of the enological strains used, we hypothesized that they may differ in their sorption capacities. We cultured all strains in synthetic must (see Yeast and Culture Medium) and harvested cells at the end of the alcoholic fermentation. ADY is usually employed for alcoholic fermentation in winemaking. These yeast forms are typically produced by the industrial process of spray-drying, facilitating easy storage and transport of enological yeast strains. We therefore also studied the sorption capacity of three ADY strains (*S1*, *S2*, and *S3*) after rehydratation. Statistical analysis of sorption capacity using the ANOVA test (Tukey test) revealed significant differences (10 groups) between the different modalities (**Table 3**). The largest values obtained for 4-ethylphenol sorption capacity were 1.697 mg m^{-2} (±0.027 mg m^{-2}) for the *S4* strain after alcoholic fermentation in synthetic must and 0.158 mg m^{-2} (±0.010 mg m^{-2}) for the *S1* strain after YPD culture (**Table 3**). Significant differences in 4-ethylphenol sorption capacity were observed between strains subjected to the same conditions of biomass production: values obtained for the ADY forms clustered into three significant groups; cells harvested after alcoholic fermentation (AF) could be clustered into five significant groups; and cells cultured in YPD medium formed three groups (**Table 3**). Values expressed as ratios of the highest sorption capacity to the lowest sorption capacity obtained for each culture method showed the greatest effect of strain on 4-ethylphenol sorption: 1.55 for ADY, 3.50 for AF, and 2.75 for YPD cultures. Significant differences in sorption capacity also existed between cells of the same yeast strain submitted to different culture conditions. For all strains studied, the sorption capacity of 4-ethylphenol was significantly higher for biomass obtained from alcoholic fermentation than for biomass from YPD culture. Active dry yeasts also have a greater sorption capacity than biomass obtained from alcoholic fermentation or YPD, even if the effect is limited in the *S3* strain case.

Table 4. Sorption Capacities of Mutant Strains Cultured in YPD Medium[a]

strain	4-ethylphenol sorption capacity (mg m^{-2})
BY4741	0.340 b ± 0.010
YIR019c (Δ *gas1*)	0.053 f ± 0.003
YBR067c (Δ *fks1*)	0.321 bc ± 0.031
YKL096w (Δ *muc1*)	0.303 d ± 0.009
YGR229c (Δ *tip1*)	0.499 a ± 0.008
YLR342w (Δ *cwp1*)	0.326 bc ± 0.004
YMR307w (Δ *smi1*)	0.289 e ± 0.010
YJL062w (Δ *las21*)	0.288 e ± 0.004
YFL014w (Δ *hsp12*)	0.516 a ± 0.005

[a] Values followed by different letters are significantly different ($p < 0.05$) by the ANOVA test.

To determine key compounds of the yeast wall involved in this process, the 4-ethylphenol sorption capacities of eight *S. cerevisiae* mutant strains were studied in the same conditions after culture in YPD (see Yeast and Culture Medium). These laboratory mutant strains are not adapted to media containing high levels of sugar, such as synthetic must, and thus were only tested after YPD culture. These mutants were each deleted for a gene encoding for a protein involved in the yeast wall structure (**Table 1**). The proteins can be classified in two groups according to their function: structural mannoproteins (Muc1p, Cwp1p, and Tip1p) and proteins with enzymatic activity localized on the cell wall or on the external face of the plasma membrane (Gas1p, Smi1p, Fks1p, Las21p, and Hsp12p). Thus, the surface proteins selected were involved in different elements of yeast wall structure and composition, representing a wide range of protein localization sites, biochemical composition, and function. Using the ANOVA test on sorption, the sorption capacity values of the nine strains could be clustered into five significant groups (**Table 4**). Three strains, including the wild type strain, had similar 4-ethylphenol sorption rates (group b). The absence (or reduced levels) of the *Fks1*- and *Cwp1*-encoded proteins did not have a significant effect on 4-ethylphenol sorption by yeast. Values measured for strains with *muc1*, *smi1*, or *las21* deletion were significantly lower than that of wild type [decreases of 11% (*muc1*), 16.1% (*smi1*), and 16.4% (*las21*)] (**Table 4**). Two mutant strains, with *hsp12* and *tip1* gene deletions, had a significantly higher sorption capacity than the wild type strain (51.5 and 41.5% higher, respectively) (**Table 4**). The largest decrease in sorption capacity was observed for the mutant

Table 5. Surface Physicochemical Properties of the Studied Strains as a Function of Biomass Production Method[a]

	strain	hydrophobicity (%)	zeta potential (mV)	electron donor character (%)
ADY	*S1*	86.78 b ± 0.6	−8.89 b ± 0.27	8.48 fgh ± 2.90
	S2	29.22 f ± 4.2	−8.37 b ± 0.10	18.72 def ± 1.78
	S3	58.05 d ± 1.2	−13.49 fg ± 0.22	0.09 hi ± 0
AF	*S1*	50.16 de ± 2.2	−14.01 fgh ± 0.59	42.15 ab ± 3.81
	S2	30.45 f ± 2.1	−5.74 a ± 0.06	26.03 cd ± 3.91
	S3	15.12 g ± 1.6	−14.16 fgh ± 0.34	12.83 efg ± 0.57
	S4	87.74 b ± 1.4	−13.97 fgh ± 0.42	7.69 gh ± 3.22
	S5	25.25 f ± 1.7	−16.69 l ± 0.20	47.97 a ± 6.92
	S6	98.31 a ± 0.3	−14.86 gh ± 0.57	3.51 ghi ± 2.25
	S7	52.67 de ± 2.9	−14.99 gh ± 0.74	32.68 bc ± 5.21
YPD	*S1*	74.94 c ± 1.8	−10.68 cd ± 0.35	48.13 a ± 6.42
	S2	48.35 e ± 3.2	−11.62 de ± 0.10	5.60 ghi ± 2.15
	S3	31.01 f ± 4.6	−10.93 cd ± 0.32	0.02 hi ± 0.01
	S4	91.58 ab ± 4.2	−12.83 ef ± 0.84	−4.48 l ± 2.29
	S5	26.80 f ± 4.5	−9.77 bc ± 0.77	46.78 a ± 5.16
	S6	94.89 ab ± 0.9	−13.53 fg ± 0.45	6.20 ghi ± 0.78
	S7	54.34 de ± 4.3	−15.36 hi ± 0.92	21.83 de ± 2.11

[a] ADY, biomass obtained after hydration of the active dry yeast; AF, biomass obtained after alcoholic fermentation in synthetic must; YPD, biomass obtained after culture in YPD medium. Values followed by different letters are significantly different ($p < 0.05$) by the ANOVA test.

Table 6. Surface Physicochemical Properties of the Mutant Strains[a]

strain	hydrophobicity (%)	zeta potential (mV)	electron donor character (%)
BY4741	34.34 bc ± 7.59	−18.06 cd ± 0.72	20.28 b ± 3.24
YIR019c (Δ *gas1*)	32.12 bcd ± 5.04	−15.64 bc ± 1.34	32.17 a ± 1.21
YBR067c (Δ *fks1*)	17.26 de ± 4.19	−14.47 b ± 1.32	15.88 b ± 1.69
YKL096w (Δ *muc1*)	44.94 ab ± 5.97	−6.51 a ± 1.12	29.65 a ± 1.26
YGR229c (Δ *tip1*)	52.31 a ± 7.49	−16.52 bc ± 1.46	18.06 b ± 3.61
YLR342w (Δ *cwp1*)	28.80 cd ± 0	−15.04 bc ± 0.87	30.30 a ± 4.08
YMR307w (Δ *smi1*)	11.65 e ± 5.23	−14.22 b ± 1.21	15.18 b ± 1.37
YJL062w (Δ *las21*)	26.03 cde ± 5.34	−8.96 a ± 0.41	28.08 a ± 1.18
YFL014w (Δ *hsp12*)	46.51 ab ± 1.712	−20.05 d ± 0.67	17.91 b ± 1.08

[a] Values followed by different letters are significantly different ($p < 0.05$) by the ANOVA test.

carrying a deletion of the Gas1p-encoding gene, with a value for 4-ethylphenol sorption 74.4% lower than wild type (**Table 4**).

Surface hydrophobicity, electron donor character, and zeta potential were measured for each strain and for each method of biomass production (**Tables 5** and **6**). These physicochemical parameters are often studied to determine the mechanisms involved in cell−cell adhesion or cell−material adhesion. Our findings are consistent with previous studies: generally, *S. cerevisiae* strains are characterized principally by electron donor character (*15*), negative zeta potential with an isoelectric point around 2.5 (*17*), and varying hydrophobicity or hydrophilic properties, classified into two groups (type I, hydrophilic strains; type II, hydrophobic strains). Most yeast cells tend to be classified as hydrophilic (**Tables 5** and **6**).

We found significant differences in surface hydrophobicity, charge, and electron donor character between biomass of cells from the same strain cultured in different media (YPD medium or synthetic must) or subjected to an industrial drying process (ADY) (**Table 5**). In terms of hydrophobicity the *S1* and *S3* strains are distributed in three groups determined by the ANOVA test and two groups for the *S2* strain. The *S1* and *S2* strain values of zeta potential are also distributed in three different groups and two for the *S3* and *S5* strain values. According to the electron donor measurements, the *S1*, *S2*, *S3*, *S4*, and *S7* strain electron donor characters are distributed in two groups. Surface hydrophobicity and zeta potential of the *S1* and *S3* strains were increased in cells exposed to ADY treatment. Cells (with the exception of *S2*) grown in YPD medium exhibited greater hydrophobicity and a larger zeta potential than cells of the same strain harvested after alcoholic fermentation, but similar electron donor character.

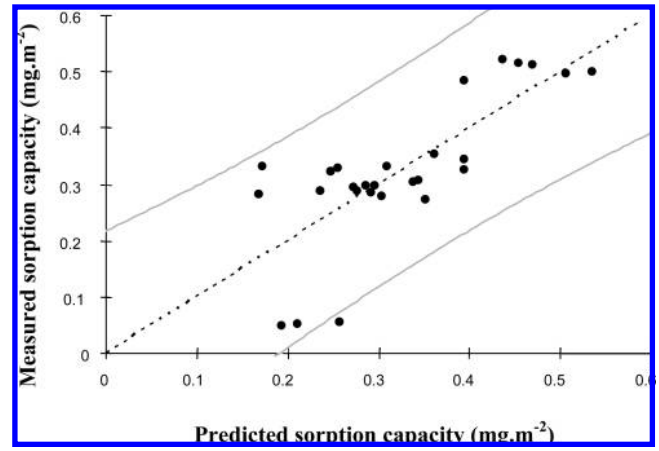

Figure 2. Correlation between measured 4-ethylpenol capacity and 4-ethylphenol sorption capacity predicted by the multiple linear regression model. Model: predicted sorption capacity (mg m^{-2}) = $4.11 \times 10^{-1} + 4.91 \times 10^{-3}$[surface hydrophobicity (%)] − 1.11×10^{-2} [electron donor character (%)].

A narrower distribution of hydrophobicity and electron donor character of mutant strains than for enological strains was observed (**Tables 5** and **6**). For the same condition of growth (YPD medium), the ANOVA test determined four different groups for the zeta potential and two for the electron donor character, whereas for these physicochemical parameters the differences between the enological strains were higher, with seven different groups for the zeta potential and five for the electron donor character. The surface hydrophobicity, electron donor character, and zeta potential of all laboratory strains were markedly lower than those of the *S1*, *S2*, *S3*, *S4*, *S4*, *S5*, *S6*, and *S7* strains subjected to the same conditions of biomass production (**Table 6**) with the exception of *muc1* and *las21* deletions. In our study conditions, these laboratory strains had hydrophilic surface characteristics with a low electron donor character.

Modelization of 4-ethylphenol sorption capacity according to physicochemical properties of the cell surface was determined using multiple linear regressions. This study on enological strains demonstrated a nonsignificant effect (significant difference at $p < 0.05$) for the three physicochemical factors studied (surface hydrophobicity, electron donor character, and zeta potential) on 4-ethylphenol sorption. By contrast, using the same statistical analysis applied to the mutant strains data, we found a mathematical model of sorption prediction (**Figure 2**). In this model, surface hydrophobicity had a positive influence, whereas electron donor character influence was negative and the zeta potential parameter was still nonsignificant on 4-ethylphenol sorption by yeasts. Nevertheless, the correlation coefficient between the predicted sorption capacities, calculated with the model obtained from analysis, and the 4-ethylphenol sorption capacities measured was quite low ($R^2 = 0.588$) (**Figure 2**).

DISCUSSION

Variations in sorption capacities observed for the enological strains (**Table 3**) may reflect differences in cell-wall composi-

tion. Indeed, it is known that cell-wall composition varies greatly between different yeast strains grown in the same conditions (*13*). In the case of the differences in sorption capacities observed for a given strain cultivated in different culture media (**Table 3**), we could also link these observations to variations in the yeast wall compositions and structures: the nature and composition of the growth medium and the culture conditions have significant effects on yeast wall structure and composition (*13*). Heat treatment used to dry the yeast causes significant changes in the parietal structure: changes in glucan microstructure and properties (*18*) and denaturation of proteins and enzymes are consequences of the industrial drying methods used to produce ADY forms. Our findings show that the sorption capacity of 4-ethylphenol by yeasts is greatly influenced by (i) strain nature, (ii) methods and medium used for cell culture before contact with the volatile phenol, and (iii) drying after harvesting. This is the first demonstration of the effect of yeast strain and growth medium on 4-ethylphenol sorption capacity. However, it is impossible to quantitatively predict an increase or a decrease in 4-ethylphenol sorption capacity for cells grown in a given medium, because the change in sorption capacity over time seems to be strain-specific, with cells in each strain reacting differently as a function of external factors. This improves the fact that the main factor studied is the nature of the strain. Nevertheless, an approach based on the selection of strains and culture methods, together with careful assessment of the drying methods used, may be beneficial to optimize the sorption of small molecules in wine.

The values for sorption capacities obtained from mutant strains were compared to those from the wild type strain to confirm an effect of the absence of these proteins on 4-ethylphenol sorption. According to the results (**Table 4**), the lack of Gas1p induces an important decrease of 4-ethylphenol sorption by yeast cells. This protein may directly or indirectly affect 4-ethylphenol sorption. Indeed, Gas1p is both a structural mannoprotein and a functional enzyme involved in the synthesis of the β-1,3-glucan yeast wall matrix (*19*). Gas1p may form 4-ethylphenol binding sites, and/or its enzymatic activity in glucan matrix polymerization may play an important role in the sorption process. However, the glucan matrix seems to have limited effect on 4-ethylphenol sorption. Indeed, values measured for the mutant lacking Fks1p—a protein involved in β-1,3-glucan synthesis (*20*)—were similar to the sorption capacity of the wild type (**Table 4**). Moreover, the Δ*smi1* mutant strain also has a lower glucan content (*21*), and its sorption capacity is closer to that of the wild type strain (**Table 4**) than to that of the Δ*gas1* mutant strain. The deletion of the gene encoding Gas1p induces increased production of chitin and certain mannoproteins including Cwp1p (*22*). The Δ*cwp1* strain had a sorption capacity similar to that of the wild-type strain, suggesting that Cwp1p has limited influence on 4-ethylphenol sorption (**Table 4**). The level of chitin in the cell wall also seems to have limited effect on 4-ethylphenol sorption. Indeed, the Δ*fks1* mutant strain, for which chitin levels are 6 times higher than for wild type (*23*), had a 4-ethylphenol sorption capacity similar to that of the wild type. Changes in yeast cell-wall composition resulted in different sorption capacities: the cell-wall mannoproteins seem to play a more important role than the glucan matrix or chitin in 4-ethylphenol sorption. The fact that the Δ*las21* and Δ*gas1* mutants have a lower 4-ethylphenol sorption capacity than wild type (**Table 4**) is consistent with this, given that these mutant strains release large amounts of mannoproteins into the medium (*24*). We eliminated this soluble fraction of mannoproteins by the three steps of biomass washing before the addition of 4-ethylphenol. The important role of mannoproteins in wine aroma retention was previously demonstrated by Lubbers et al. (*25*) for isolated yeast mannoproteins. Our findings provide further insight, highlighting the positive effect on this process of Gas1 protein and the negative effects of the presence of Tip1p and Hsp12p in the yeast wall. This study has demonstrated the involvement of three particular surface proteins in 4-ethylphenol sorption: Hsp12p, Tip1p, and Gas1p. Hsp12p and Tip1p seem to decrease the number of potential 4-ethylphenol binding sites. The mannoprotein Gas1p, however, seems to play a positive role in this sorption process. Moreover, we showed that, despite mannoproteins representing a major component of the yeast wall, not all mannoproteins seem to contribute to 4-ethylphenol sorption. However, determination of the real effect of the absence of a mannoprotein on 4-ethylphenol sorption by the yeast wall is complex due to the presence of compensatory mechanisms of resistance. Increased glucan and chitin levels in the mutant strain cell walls do not have a positive effect on volatile phenol sorption. This supports the notion that the mannoprotein fraction of the yeast wall is directly involved in 4-ethylphenol sorption. We studied a number of gene deletion mutants. It is likely, however, that the expression levels of other genes involved in cell-wall synthesis also affect the sorption capacity of the yeast wall.

Changes in cell-surface physicochemical properties of the enological strains (**Table 5**) as a function of the medium and the methods of culture are also the result of changes in yeast wall composition (*13*). Yeast cells exhibit a strain-specific response to growth conditions: physicochemical properties differ between strains even if the methods and media used for culture are similar (*14*). Differences in surface physicochemical properties can be correlated to variations in yeast wall biochemical composition caused by cell growth conditions for enological strains. Culture in synthetic must medium exposes the yeast cells to various stresses. In particular, ethanol stress occurs during alcoholic fermentation. Rossignol et al. (*26*) demonstrated a large effect for alcoholic fermentation and stationary phase entry on gene expression in the cell wall in *S. cerevisiae*: specific genes encoding proteins involved in cell-wall biosynthesis are strongly up-regulated at the end of alcoholic fermentation (*fks1*, *gsc2*, *ssd1*, *mpt5*). This specific response to the synthetic must medium may underlay the differences observed in surface physicochemical properties and the sorption capacity between cells cultivated in YPD medium and synthetic must. Indeed, the surface charge of yeast cell walls is accounted for by specific chemical groups such as polypeptide amino groups and phosphate groups of mannans, which are fully dissociated at the tested pH (3.5) (*27*), or carboxylic groups. Surface hydrophobicity is correlated with the N/P surface concentration ratio (*28*) and with the presence of specific mannoproteins such as Muc1p (*29*) and Hsp12p (*30*). The level of these different elements is closely dependent on medium composition and nutrients availability. On the contrary, the increased surface hydrophobicity of ADY cells could be due to protein denaturation (*31*), but it is not correlated with an increase of H bond donor availability (*18*) caused by the heating process during drying.

Concerning mutant strains, the deletion of one mannoprotein gene induced perceptible changes in surface physicochemical properties (**Table 6**) by modifications of yeast wall mannoproteins level and by the lack of their enzymatic function on yeast wall. Indeed, adhesion studies in pathogenic yeast species such as *Candida albicans* have shown that exposed mannoproteins have a significant effect on cell adhesion in tissues due to high surface hydrophobicity (*12*). It is therefore possible that the

differences observed in sorption capacities correspond to specific changes in surface physicochemical properties caused by a lack of these surface proteins.

The differences observed in surface physicochemical properties between enological and laboratory strains (**Table 5** and **6**) could be due to the fact that the mutant strains used are very different in terms of their physiology and genotype: parietal composition and structure may thus be very different. The cell surface of enological strains has to adapt to a number of environmental factors (high level of ethanol, acid pH) and protect their intracellular integrity.

We gained further insight into the nature of the interaction involved in 4-ethylphenol sorption by studying the correlation between sorption capacity and the physicochemical surface properties of yeasts. The absence of correlation between predicted and experimental sorption capacities among the enological strains studied could be explained by the fact that the ranges of the physicochemical values were higher for these strains than for the mutant strain. We can also suggest that the lack of correlation could be explained by the fact that the methods used for yeast surface characterization give only global information on a yeast population. Nevertheless, the correlation between 4-ethylphenol sorption capacity and the physicochemical properties of the mutant strains (**Figure 2**) is consistent with a major role for mannoproteins in 4-ethylphenol sorption. We noted that the greater surface hydrophobicity of mutant strains significantly enhanced 4-ethylphenol sorption capacity, whereas the greater electron donor capacity of these mutants resulted in significantly lower 4-ethylphenol sorption capacity. Over the hydrophobicity range studied, we found that the two mutants with both the lowest electron donor capacity and the least hydrophilic character had the highest sorption capacity (Δ*hsp12* and Δ*tip1* strains). By contrast, increased electron donor capacity seemed to decrease the sorption capacity in strains with similar hydrophilic characters; this was observed for Δ*gas1* strain, which had a similar hydrophilic character but higher electron donor capacity than wild type. Thus, interactions other than hydrophobic interactions seem to be involved in this sorption, as previously demonstrated for 4-ethylphenol sorption by lees (*9*). However, the quite low regression coefficient ($R^2 = 0.588$) (**Figure 2**) indicates that surface hydrophobicity and electron donor character do not fully account for the variation observed in 4-ethylphenol sorption. Moreover, surface hydrophobicity was not the main determinant of 4-ethylphenol sorption capacity. The apparent coupling effect of surface hydrophobicity and electron donor character on sorption in yeast indicated that the underlying mechanisms involved a balance between different kinds of interactions. This may reflect the presence of multiple 4-ethylphenol binding sites, exhibiting different properties on the yeast wall.

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