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Surface properties of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*: chemical composition, electrostatic charge and hydrophobicity

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Key words: Cell surface; Chemical composition; Surface charge; Hydrophobicity; (*S. cerevisiae*); (*S. carlsbergensis*)

The surface composition of nine top and bottom fermentation brewery strains was determined by X-ray photoelectron spectroscopy. Individual strains differ mainly in the minor constituents phosphate, ammonium and potassium. These surface concentrations are approximately twice as high for the bottom strains as compared to the top strains. The surface charge was studied by electrophoretic mobility. At pH 4, the bottom strains are more negative (-35 ± 5 mV) than the top strains (-12 ± 9 mV). The zeta potential at pH 4 is correlated with the phosphate surface concentration. There is a linear 1 to 1 relationship of phosphate with potassium and with ammonium, indicating that these cations are the main counterions of the cell surface. Phosphates play a predominant role in the development of the surface charge and the participation of carboxylic groups is minor. The hydrophobicity, estimated by the water contact angle, is correlated with the N/P surface concentration ratio. The bottom strains are less hydrophobic than the top strains. Surface charge and hydrophobicity could account for the behaviour of top and bottom strains in fermentation. The study of a non-brewing strain (*Saccharomyces cerevisiae*) shows that the systematic differences of surface properties found between top and bottom fermentation strains cannot be generalised to *S. cerevisiae* and *Saccharomyces carlsbergensis*.

Introduction

In biotechnology and microbiology, various processes rely on the association of cells with themselves (flocculation), with foreign cells (coimmobilization, infections), or with an inert material (adhesion, biocompatibility, fouling) [1–3]. The importance of the cell surfaces in these events is obvious. However, the knowledge of biological surfaces is still in its infancy, due to the complexity of cell surfaces and to the lack of methods which provide a surface specific information.

X-ray photoelectron spectroscopy (XPS) is a powerful technique for the study of surfaces. The sample is irradiated by an X-ray beam which induces the ejection of electrons. The kinetic energy of the photoelectrons is analysed; it permits the calculation of their binding energy. Each peak is characteristic of a given energy level of a given element. Due to unelastic scattering of the electrons in a solid, the information collected concerns specifically the outermost molecular layers (less than 10 nm) of a surface. Shifts in the peak binding energy permit the identification of the bonds or even the functions in which the elements

Abbreviations: XPS, X-ray photoelectron spectroscopy; HIC, hydrophobic interaction chromatography.

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are involved [4]. In addition to its surface specificity, the information obtained does not have to be interpreted in the frame of a theoretical model, like microelectrophoresis for instance.

Few attempts have been made to apply XPS to biological surfaces [5–10] and the studies remained qualitative. We have demonstrated [11] the reliability and the consistency of the XPS analysis of yeast cells. In particular it has been shown that the dehydrated surface probed by XPS is intimately related to the hydrated surface characterized by electrophoretic mobility measurements.

This paper is dedicated to a surface study of various yeast strains of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*. Global surface properties of the whole cells, the electrostatic charge and the hydrophobicity are tentatively related to the surface chemical composition. Correlations between surface properties and yeast behaviour in beer fermentation are also examined: two important features of brewery strains are their tendency to flocculate and whether or not they gather at the top or at the bottom of the medium at the end of the fermentation process; top fermentation and bottom fermentation strains are *S. cerevisiae* and *S. carlsbergensis*, respectively.

Materials and Methods

Cells

The following strains were studied: *Saccharomyces cerevisiae*, non brewing: D273-10B (Dr. Tzagoloff, Columbia University, New York); *S. cerevisiae*, brewing: 28733, 28734, 28360, 28788 and 28787; *S. carlsbergensis*, brewing: 28285, 28292, 28286 and 28304. The brewery strains were supplied by the Laboratory of Brewery (UCL, Belgium) and belonged to the MUCL collection (UCL, Belgium).

The strains were grown in the following medium: 20 $g \cdot l^{-1}$ yeast extract, 50 $g \cdot l^{-1}$ glucose. They were collected after 24 h growth (beginning of the stationary phase) at 30 °C and washed three times in distilled water. For XPS analysis and contact angle measurements, the freshly harvested cells were frozen at liquid nitrogen temperature, freeze-dried at −5 °C in a Lyovac GT4 (Leybold Heraeus), and stored in a dessicator.

XPS analyses

The XPS analyses were executed with a Vacuum Generators ESCA 3 MK 2 spectrometer. The residual pressure in the spectrometer was in the range $1–5 \cdot 10^{-9}$ torr. The samples were mounted in stainless steel troughs and pressed in order to obtain smooth surfaces. Five samples of yeast were introduced together and analysed in the same XPS experiment. One sample of a pure silica powder was added as an external standard [11].

Usually, the quantitative information provided by XPS is expressed as atomic concentration ratios with respect to a given element. In this work the intensity of the Si_{2p} peak, I_{Si2p}, of the silica sample included in each run has been used to normalize the intensities I_{Xy} of the yeast samples. This made it possible to determine apparent surface concentrations [X]′ by relation

$$[X]' = \frac{I_{Xy} \cdot i_{Si2p} \cdot [Si]}{I_{Si2p} \cdot i_{Xy}}$$

where i_{Si2p} and i_{Xy} are sensitivity factors [12] and [Si] is the atomic concentration of Si in silica (39 $mmol \cdot cm^{-3}$). The apparent surface concentrations [X]′ are estimates of true concentrations (mol/volume) in the surface zone because the surface roughness and the electron mean free path for the yeast samples and the silica standard may be different [11]. The surface concentrations may thus be affected by a systematic error; however, they can be used safely in comparisons.

The carbon and nitrogen peaks were decomposed into three and two components, respectively; a constant full width at half maximum was imposed for the components of a given peak; the binding energies and intensities were determined with a best-fit program.

Contact angle measurement

The hydrophobicity of the cells was characterized by the measurement of the water contact angle. The freeze-dried cell powder was pressed into a coherent layer which was then attached to a microscope slide by a double-faced adhesive tape. The test consisted of depositing a drop of water ($0.5 \cdot 10^{-6}$ l) on the sample and measuring the angle between the surface and the tangent to the drop at the solid-liquid-air meeting point. This

method has been described in detail by Mozes and Rouxhet [13].

Each determination involved measurements on at least 10 drops.

Hydrophobic interaction chromatography (HIC)

The hydrophobicity was also estimated by HIC. It consisted of measuring the amount of cells retained by an hydrophobic gel (Phenyl-Sepharose from Pharmacia). The method has been described in detail elsewhere [13]. The suspension medium was 0.6 M NH_4Cl; 0.1 ml of a cell suspension (about $125 \cdot 10^{-6}$ cells $\cdot$ ml^{-1}) was deposited on the top of the gel and eluted with 3 ml of the suspension medium. The percent retention was determined by measuring the amount of cells eluted ($A = 800$ nm).

Microelectrophoresis

The electrophoretic mobility of the cells was determined with a Laser Zetameter (model 500) of Pen Kem Inc. It was converted into zeta potential on the basis of the Smoluchowski equation. The measurements were performed on freshly harvested cells, washed three times and resuspended in distilled water. The final cell concentration was in the range $0.5–1 \cdot 10^6$ cell $\cdot$ ml^{-1}. The pH was adjusted by HCl or NaOH; the ionic strength was not adjusted by addition of any other reagent. The suspensions were agitated upside-down (60 rpm) at 20 °C for 1 h. The pH was measured and electrophoretic mobility determinations were performed. The particle mobility profile through the electrophoresis chamber was determined in order to check that the flow pattern was parabolic and that there was no perturbation arising from cell sedimentation. For some strains, avoiding perturbation by sedimentation required vertical mounting of the electrophoresis cell.

Atomic absorption spectroscopy

The calcium and potassium analyses were performed with a Varian Techtron AA5 spectrometer.

Results

Surface composition

The XPS analysis of the nine brewery strains was performed on at least two independent cultures and preparations. Table I presents the binding energy and the full width at half maximum of the various peaks and of the components of C_{1s} and N_{1s} peaks. In most cases, the individual values of the various parameters were not significantly different for the nine strains; consequently, mean values are reported. The latter are similar to those of *Saccharomyces cerevisiae*, strain D273-10B [11].

The three components of C_{1s} peaks are due, respectively, to carbon involved in C–C and C–H bonds (285.0 eV), in C–O bonds (286.4 eV), and in the amide bond (288.1 eV). It should be noted that the decomposition procedure does not permit the identification of components smaller than 5%; therefore, carbon of carboxyl groups is not observed.

Oxygen appears as a simple peak attributed to the C–O bond. The N_{1s} peak is accounted for by the amide bond (400.2 eV) and by ammonium ions or protonated amines (402.1 eV). The mean value of the relative intensity of the components in C_{1s} and N_{1s} peaks is given in Table I; however, there are significant differences between individual strains, which will be commented on below. Phosphorus, present as phosphate, and potassium were detected at a lower level.

The apparent surface concentrations for the nine brewery strains are presented in Table II. The results of strain D273-10B are presented for comparison.

The mean O/C ratio for all the brewery strains is 0.30. As the cell wall is composed mainly of polysaccharides, one would expect a ratio close to 1. If one also considers the very low mean N/C ratio (0.02) which indicates that proteins are a minor constituent of the surface, the very low O/C ratio can only be explained by the predominance of lipids or hydrocarbonaceous substituents at the cell surface; for the strain D273-10B, a proportion of 68% had been estimated [11]. A similar estimation for the nine brewery strains leads to a mean proportion of $76 \pm 2\%$. Differences of C and O concentrations between the top and bottom fermentation yeasts are characterized by a level of significance of 95% for C and 99% for O. However, the differences are relatively small and may be strongly influenced by differences in the surface roughness of the cell

preparation, due to cell size or aggregation. The level of significance of the difference between the two types of yeasts is 99% for K and 99.9% for P and for the N/P ratio.

The case of N deserves more detailed comments. Although there is no significant difference in total N concentration between the two types of cells, there is a difference ($P = 0.99$) in the proportion of the component due to protonated nitrogen: $8.5 \pm 3.5\%$ for the top fermentations yeasts, and $16.4 \pm 4.5\%$ for the bottom fermentation yeasts.

Fig. 1A shows the correlation between the potassium and phosphorus apparent surface concentrations. This strongly suggests that the potassium cations are retained by a mechanism of ion exchange on the phosphate groups of the cell surface and are the main counterions.

Freshly harvested cells of strain D273-10B have been washed and stored at 4°C during 3 days as a suspension and as a pellet. One portion of each

TABLE I

CHARACTERISTICS OF XPS PEAKS OF TOP AND BOTTOM FERMENTATION BREWERY STRAINS (MEAN FOR NINE STRAINS)

Identification of the peak, binding energy (E_b), identification of the functions, full width at half maximum (FWHM) of the peak, proportion of the various components.

Element (peak)	Component			
	E_b (eV)	functions	FWHM (eV)	proportion (%)
$C_{(1S)}$	285.0 *	$-CH_2-CH_2-$	2.15 *	49 ± 4
	286.4 ± 0.1	-C-O-	2.15 *	39 ± 2
	288.1 ± 0.1	-(C=O)-NH-	2.15 *	12 ± 2
			3.2 ± 0.1 **	100
$O_{(1S)}$	533.3 ± 0.1	-C-O-	2.6 ± 0.1	100
$N_{(1S)}$	400.2 ± 0.1	-(C=O)-NH-	2.4 *	88 ± 6
	402.1 ± 0.3	NH_4^+	2.4 *	12 ± 6
			2.6 ± 0.1 **	100
$P_{(2P)}$	133.6 ± 0.1	$-PO_4^-$	2.6 ± 0.1	100
K_{2S}	377.8 ± 0.2	K^+	3.8 ± 0.2	100

* Imposed.

** For the whole peak.

TABLE II

SURFACE PROPERTIES OF BREWERY STRAINS (TOP AND BOTTOM FERMENTATION) AND STRAIN D273-10B

Strains	Apparent surface concentration [a] ($mmol \cdot cm^{-3}$)					Surface concentration ratio N/P	Hydrophobic interaction chromatography (% retention) [b]	Contact angle [c]	Zeta potential at pH 4 [d] (mV)
	C	O	N	P	K				
S. carlsbergensis									
Bottom fermentation									
MUCL 28285	63.7	19.0	1.2	0.17	0.22	7.0	94	64	−34
28292	65.7	18.9	1.3	0.20	0.24	6.4	45	52	−29
28286	66.8	19.0	1.5	0.23	0.33	6.4	38	65	−40
28304	62.6	17.6	1.4	0.22	0.26	6.3	65	57	−38
S. cerevisiae									
Top fermentation									
MUCL 28733	69.3	20.6	1.8	0.13	0.14	15.4	*	79	−7
28734	72.5	20.8	2.0	0.09	0.09	23.4	*	90	−19
28360	65.8	22.2	1.0	0.09	0.18	12.7	27	74	−24
28788	66.1	20.2	1.4	0.10	0.11	15.5	86	76	−6
28787	69.6	21.9	1.3	0.08	0.13	18.2	96	72	−6
Non-brewing									
D273-10B	71.6	27.8	0.9	0.20	0.26	6.5	27	58	−32

* Flocculated in the test.

[a] Mean for two cultures prepared independently and at least two XPS analyses per specimen.

[b] Mean of two simultaneous determinations on a given cell suspension.

[c] Mean for two independent cultures prepared independently and analysed by two different operators (10–15 measurements per sample).

[d] Determined on one cell suspension, mean for nine measurements at the lower stationary level and six at the upper level.

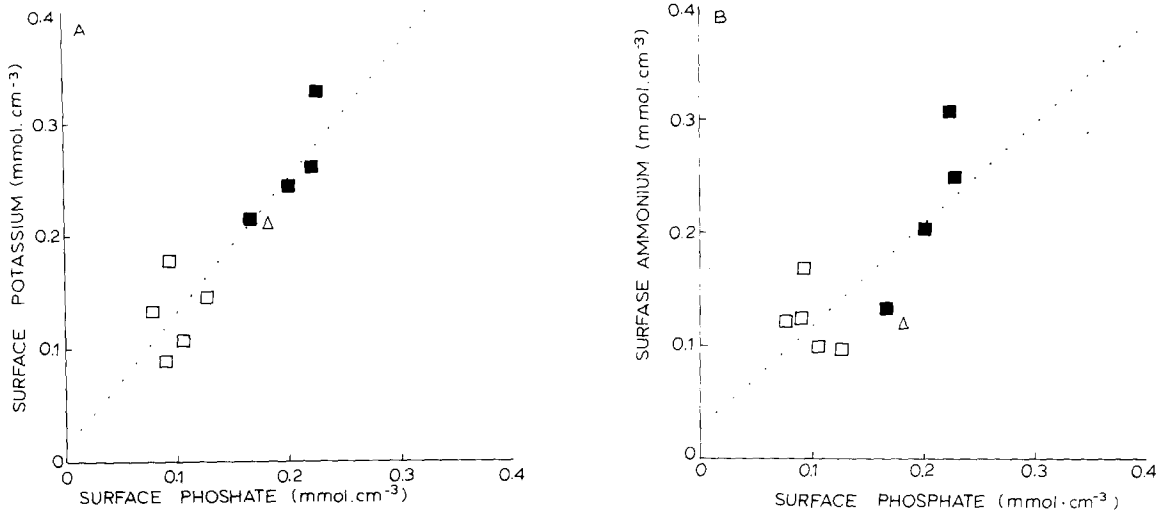

Fig. 1. Apparent surface concentrations of K^+ (A) and NH_4^+ (B) as a function of phosphate apparent surface concentration. Top fermentation strains (□), bottom fermentation strains (■), strain D273-10B (△).

was washed with water, another portion was not washed; the four samples were then submitted to XPS analysis. Aging the cells induces an increase of both the K and the P signals. The increase is larger for the cells aged as a pellet and for the cells not washed after storage. This indicates that the cells release phosphate or phosphate-containing substances which are adsorbed on the surface and have potassium as the main counterion.

In order to examine the ion exchange properties of the cell surface, the strain D273-10B has been treated with calcium ions which were expected to replace the monovalent cations of the surface. After the harvest, the cells were starved by flushing sterile air through the suspension at 20 °C for 24 h in order to minimize active transport. Then, the cells were washed again with distilled water and finally treated with $CaCl_2$ solutions in the range 0–0.5 mM; three different cell concentrations were used, 30, 160 and $420 \cdot 10^6$ cells $\cdot$ ml^{-1}, for each $CaCl_2$ solution. The suspensions were gently agitated for 30 min at 4 °C and centrifuged; the treatment was performed three times and the last pellet was freeze-dried for XPS analysis. The mean value of the $K/(2 \cdot Ca)$ surface concentration ratio was 0.5 for the $30 \cdot 10^6$ cells $\cdot$ ml^{-1} concentration and 1.7 for the 160 and $420 \cdot 10^6$ cells $\cdot$ ml^{-1} concentrations. The surface concentration ratio $Cl/(2 \cdot Ca)$ was 0.1; this shows that the contamination of the cell surface by $CaCl_2$ contained in the extracellular space of the freeze-dried pellet is negligible under these experimental conditions. Potassium was thus always present on the cell surface, whatever the cell and calcium concentration. With the concentrated suspensions, it was even the predominant ion. Such a selectivity in favour of the ion of lowest charge is in contradiction with the ion-exchange theory [14]. This might be due, on the one hand, to complexation of calcium in the supernatant by excretion products of the cells. On the other hand, the release of potassium into the solution could create a high concentration at the cell surface, thereby favouring the exchange against calcium. Both phenomena would be more important for concentrated suspensions. This shows the limits of the XPS analysis, which are imposed by the requirements of the sample preparation and by the complexity of the analyzed material.

Surface charge and hydrophobicity

The zeta potential of the brewery strains has been studied through the pH range 1–10. Fig. 2 shows typical zeta potential vs. pH curves obtained with top and bottom fermentation yeasts. The curves were very similar within each type of cell. The zeta potential curves decrease from pH 3 to pH 5, pass through a minimum around pH 5

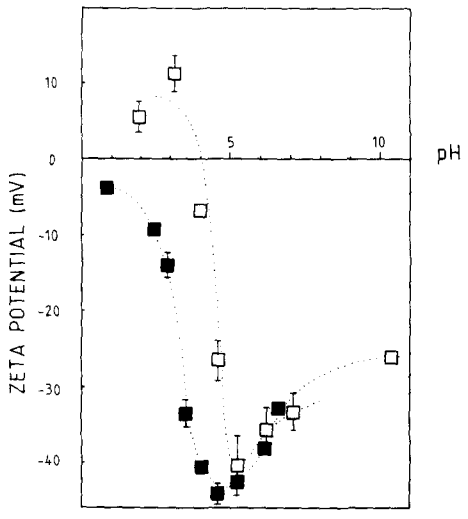

Fig. 2. Zeta potential of a top fermentation strain MUCL 28733 (□) and a bottom fermentation strain MUCL 28286 (■) as a function of pH.

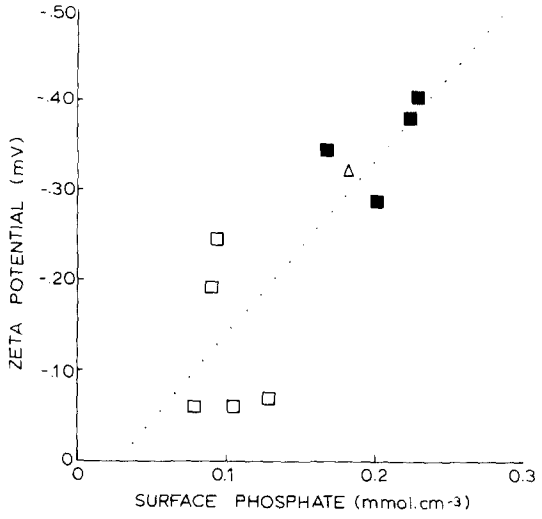

Fig. 3. Zeta potential as a function of phosphate apparent surface concentration. Top fermentation strains (□), bottom fermentation strains (■), strain D273-10B (△).

and increase again slowly up to pH 10. The curves are similar above 5 but differ from each other below pH 5. In order to characterize them, the isoelectric point was not used, as its accurate determination may require a risky extrapolation, as illustrated by strain 28286 in Fig. 2. The zeta potential at pH 4 was retained because it expressed adequately the difference between strains; moreover, this pH is reached naturally by a yeast suspension, e.g., by fermented worts in brewery. For particles of the size of yeast cells ($5 \cdot 10^{-6}$ m), the ionic strength corresponding to pH 4 is not beyond the limit of applicability of the Smoluchowski equation. The individual values for the ten strains are reported in Table II. The mean value for the top fermentation strains was significantly less negative ($P = 0.999$) than that for the bottom fermentation strains.

Fig. 3 shows the correlation ($r = -0.82$) between the zeta potential at pH 4 and the phosphate apparent surface concentration. This indicates that phosphate ions represent a constant or major proportion of the total surface sites responsible for the negative charge at pH 4. A similar relationship was observed by Eddy and Rudin [15] with the total phosphorus content of isolated cell walls of various brewery yeasts; in comparison, the relationship observed here concerns the outermost layers of the cell wall of whole cells. There is also a good correlation between the zeta potential at pH 4 and the potassium surface concentration ($r = -0.85$). This was expected as potassium is correlated with phosphates (see Fig. 1A).

There is no correlation between the zeta potential and the N surface concentration ($r = +0.32$). The correlation observed between the zeta potential and N/P ratio of the yeast cell surface ($r = +0.78$) is thus essentially due to phosphorus; actually, N does not vary as much as P between the strains.

The correlation between the zeta potential and the proportion of protonated nitrogen is negative ($r = -0.72$): a larger proportion of protonated nitrogen is related to more negative potentials. This shows that the latter groups cannot be immobile positive charges fixed on the cell surface and are rather mobile cations. These could be ammonium ions which are present in the culture media and would not be completely removed by washing. The apparent surface concentration of ammonium can be evaluated by the total N surface concentration and the proportion of protonated nitrogen. As observed for potassium, there is a positive relationship (Fig. 1B) between the ammonium and phosphate surface concentrations ($r = +0.73$). This confirms that the protonated

nitrogen detected by XPS is a mobile cation, presumably ammonium.

The hydrophobicity of the cells was approached by two techniques: contact angle and hydrophobic interaction chromatography. Contact angle was measured on a portion of the cells which were freeze-dried for XPS analysis. Table II presents the individual values for the various strains. The top fermentation strains have a systematically higher contact angle as compared to the bottom fermentation strains; the strain 28734 can be pointed out for its outstandingly high contact angle. The mean value of the contact angle of the ten strains is $69 \pm 12°$. This is significantly higher than the values obtained on air-dried cell films which is the usual technique to process the cellular material [16]. This difference is attributed to the more extensive dehydration of the freeze-dried cells. From a practical point of view, the measurements on the layer of pressed freeze-dried cells are easier and more reproducible.

Fig. 4 shows the correlation between the contact angle and the N/P surface concentration ratio ($r = +0.91$). The individual correlations were not so good: with N, $r = +0.63$ and with P, $r = -0.80$. This indicates that the hydrophobicity decreases as the surface charge imposed by the phosphate groups increases and as the N surface concentration, reflecting the protein concentration, decreases. The role of proteins in determining hydrophobicity of yeast cells was demonstrated by Kamada and Murata [17] who showed that proteolytic treatment of the cells decreased their hydrophobicity. Surprisingly, there was no correlation between the contact angle and the oxygen concentration ($r = +0.05$). However, it must be kept in mind that the variation of the apparent surface concentration was small and that, in the absence of conformational changes, hydrophobicity is determined by the first atomic layers, while XPS explores a larger depth below the surface.

The surface energy has also been approached by hydrophobic interaction chromatography (HIC). In this technique the percentage of cells which are retained on a hydrophobic gel after percolation is taken as an estimate of the hydrophobicity. Table II gives the individual values obtained on the various strains. The star indicates

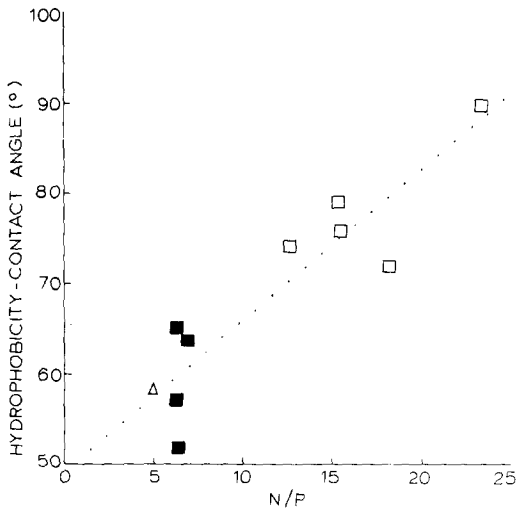

Fig. 4. Hydrophobicity estimated by contact angle as a function of the N/P surface concentration ratio. Top fermentation strains (□), bottom fermentation strains (■), strain D273-10B (△).

unvalid results due to flocculation of the cells in the suspending medium before percolation through the column. The remaining results do not show any clear trend in correlation with surface composition or distinction between top and bottom fermentation strains. However, the reproducibility of absolute HIC measurements is poor, mainly due to difficulties in obtaining homogeneous gel packing and in controlling the flow rate.

Discussion

The surface composition of yeast cells

The investigation of yeast cells by XPS provides information which is surface specific.

Comparison of atomic concentration ratios given in Table II with those of model compounds indicate that lipids (or hydrocarbonaceous substituents) could be the main components of the cell surface. Their proportion is higher than the mean proportion found in the whole cell walls of yeasts [18]. Although the results discussed below confirm that the XPS analysis is firmly related to the actual surface of a yeast cell in water [11], a surface enrichment in lipids resulting from the freeze-drying may not be excluded.

The cell surface can be adequately char-

acterized by the N/P ratio. It has been shown before [11] that the precision of its XPS determination is very good. It is correlated with the zeta potential and the hydrophobicity, which are global surface properties of the whole cells.

Potassium and ammonium are the main cations present on the cell surface; they are retained by a mechanism of ion exchange. Ammonium would be a residue of the culture medium. Potassium is the main cation of yeast cells (around 50% of the ionic content [19]). It cannot be replaced easily by calcium ions, presumably because there is a very high potassium gradient at the cell solution interphase, due to release of potassium into the solution.

The surface charge

The net charge of yeast cells is usually ascribed to side-chain carboxylic and amino groups of polypeptides together with phosphate groups in the mannans and the polyphosphates. The electrophoretic mobility or the zeta potential at pH 4 is attributed to phosphate groups [15,20]. The correlation between zeta potential at pH 4 and the phosphorus surface concentration is in complete agreement with this interpretation. The correlation suggests that there is no specific adsorption of ions. It also shows that top and bottom fermentation strains have, respectively, a low and a high surface concentration of phosphate groups, corresponding to low and high surface charge. Nevertheless, all the strains had the same zeta potential of about -40 mV at pH 5–6 which appears to be a saturation value. This is attributed to two cooperative effects [21,22]. As the surface charge becomes more negative, the resulting surface potential increases the pK_a of the remaining acid functions. On the other hand, more and more counterions are retained in the Stern layer (counterion condensation); they ensure a surface charge neutralization at a distance shorter than the shear plane where the zeta potential is measured. As a consequence, surface charges exceeding 0.05 to 0.1 $e \cdot nm^{-2}$ are not usually reflected by electrophoretic mobility [22].

The evolution of the zeta potential above pH 4 is classically attributed to deprotonation of carboxylic groups [23]. Actually, the effect of the surface charge on the apparent pK_a results probably in a weak ionisation of carboxylic groups below pH 7. James et al. [21] have calculated that the proportion of dissociated ionogenic groups with a pK_a of 5 (close to the pK_a of carboxylic residues of amino acids) is weak at pH 7; in addition it is lowered by the presence of a charge created by the dissociation of more acidic groups like sulfates. A similar effect is expected in the presence of phosphate groups. Therefore, the contribution of carboxylic groups to the surface charge cannot be evaluated by the variation of potential between pH 7 and 4 as proposed by different authors [15,24].

In the range of pH 5–10, the zeta potential is expected to decrease slightly or to remain constant if the effects mentioned above are strong enough. Actually, it is found to increase above pH 5; this is attributed to an increase of the ionic strength due to the addition of NaOH. The ionic strength was indeed imposed by the HCl or NaOH added; thereby, it was kept as low as possible in order to enhance the absolute value of the measured zeta potential and the differences between strains. Note that the pH of a cell suspension initially set between pH 7.5 and 9.5 shifts to pH 7 within 1 h.

The correlations of phosphate surface concentrations with zeta potential, on the one hand, and with surface cations K^+ and NH_4^+, on the other hand, show that phosphate groups are of primary importance with respect to the cell surface charge. This is in agreement with the work of Jayatissa and Rose [20] who showed that removal of phosphorus (HF treatment) from the wall of four yeast strains rendered the cells almost neutral.

The sum of the surface concentrations of K^+ and NH_4^+, considered as counterions, is about twice the concentration of phosphate. This discrepancy may be due to systematic errors involved in sensitivity factors and decomposition of the nitrogen peak. It is not attributed to contribution of carboxylic groups to the surface charge; the above discussion of the zeta potential curve indicates indeed that these groups are probably only weakly dissociated below pH 7; moreover, the various correlations observed with phosphate alone pass through the origin. This does not exclude the presence of carboxylic groups at the cell surface [25,26]; they were not detected by XPS because

they are too small a component of the total carbon or oxygen peak.

Comparison of yeast strains

The surface properties of the nine brewery strains examined can be summarized as follows: the bottom fermentation strains have higher phosphate, potassium and ammonium surface concentrations and appear to be systematically less hydrophobic and more negatively charged than the top fermentation strains.

The observed difference of hydrophobicity is in agreement with literature data. Eddy and Rudin [27] could distinguish top and bottom fermentation strains by their tendency to form superficial films when suspended in a small drop of water. Hinchcliffe et al. [28] confirmed this distinction by determining the partition between xylene and an aqueous phase. A higher hydrophobicity and lower surface charge should favour flocculation of the cells. It is indeed observed in brewery that top fermentation strains are almost always flocculent. On the other hand, their higher hydrophobicity accounts also for their behaviour at the end of a fermentation: it favours their association with the CO_2 bubbles and their rising to the top of the medium.

This physico-chemical investigation of brewery strains shows that top or bottom fermentation is an intrinsic characteristic of a strain, as the differences were measured on cells cultured under identical laboratory conditions. In addition to their top or bottom characteristics, the brewery strains used were announced as flocculent or non-flocculent. No correlation has been observed between this characteristic and the composition or physico-chemical properties of the surface. This is in agreement with the fact that flocculation is strongly influenced by factors which are different from those investigated here, in particular the medium composition [29] and the presence on the cell surface of compounds permitting molecular recognition interactions [30].

The conclusions concerning the difference between top and bottom strains cannot be generalised to *S. cerevisiae* and *S. carlsbergensis* strains. Effectively, the haploidic strain D273-10B, a *S. cerevisiae*, shows surface properties which are typical of bottom fermentation yeasts.

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