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On the relations between the elemental surface composition of yeasts and bacteria and their charge and hydrophobicity

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The elemental surface composition of eleven microorganisms was determined by X-ray photoelectron spectroscopy. Bacteria could be distinguished from yeasts by higher nitrogen and phosphate concentrations. Overall physico-chemical properties, electrical charge and hydrophobicity, were also investigated: the former by electrophoretic mobility measurements, the latter by contact angle and by hydrophobic interaction chromatography. Phosphate plays the major role in determining the surface electrostatic charge. A correlation is observed between the N/P atomic concentration ratio and the electrostatic charge. In bacteria, hydrophobicity is directly related to concentration of carbon in hydrocarbon form and inversely related to oxygen concentration or to the N/P ratio. For yeasts, a positive correlation is found between hydrophobicity and the N/P ratio, pointing at the role of proteins in determining the hydrophobicity.

Introduction

The bulk chemical composition of the envelopes of microorganisms has been intensively investigated [1–6]. However, information about the composition of the outermost layer (surface) of those envelopes is limited. Most of the established informations concern the topography and the nature of specific constituents; they were obtained by use of electron microscopy or specific probes (antibodies, lectins, bacteriophages) or by selective degradation of certain components.

Phenomena like flocculation (association of microorganisms among themselves), infection (interactions with animal cells) adhesion and fouling (attachment to inert support) are governed by the physico-chemical properties of the surface of the cells. The characterization of these properties raises the question of the definition of a surface, which may be difficult even for rigid and smooth bodies and much more so for cells with their complex architecture. The presence of polymers that are associated with the wall without being covalently bound may be transient, in the process of excretion into the external environment; they may extend the formal boundary of the cell wall as a 'gradient of polymers'. Cell appendages such as flagellae, pili, fimbriae, etc., many of which are not strictly cell wall components nevertheless protrude from the wall and also contribute to the complexity of the bacterial cell surface [1].

X-ray photoelectron spectroscopy (XPS) provides an elemental analysis of the outermost layer (2–5 nm) of a surface; the position and shape of the peaks give information on the bonds and thus on the chemical functions in which the elements are involved. Baddiley et al. [7] reported the use of

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

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XPS analysis for the study of bacteria, but there have been only few applications since then. Millard et al. [8] compared surface composition of Gram-positive and Gram-negative bacteria. Van Haecht et al. [9] found a correlation between the isoelectric point of various Saccharomyces yeasts and the N/P atomic concentration ratio determined by XPS. Amory et al. [10] developed a sample preparation and analysis procedure for yeast strains that minimizes contamination and re-arrangement of the surface. A correlation obtained between the surface N/P ratio and the electrophoretic mobility at pH 4 gave confidence on the reliability of the XPS analysis. A similar correlation was demonstrated for various microorganisms [11]. Examination of brewery yeasts showed significant relationships between surface composition, physico-chemical properties, flocculence, species and culture conditions [12,13].

The net surface electrostatic charge of microorganisms is investigated generally by measurement of their electrophoretic mobility. The information obtained allows to calculate the zeta potential of the cells and to define their isoelectric point.

A great interest in studying hydrophobicity of cells evolved in the last decade. Rosenberg and Kjelleberg [14] reviewed the various methods used for its evaluation. Three recent, independent studies dealing with comparison of different methods for assaying bacterial hydrophobicity [15–17] pointed at the fact that different techniques may measure different properties. Nice correlations between methods were obtained only for homogeneous sets of strains [17]. A common conclusion of those studies is that a single method is not reliable and combination of methods is recommended.

The aim of this work is to analyse the surface of various microorganisms by XPS and to examine the relationships between the chemical composition and the physico-chemical properties which are of high importance in cell-solid and cell-cell interactions, namely the electrostatic charge and the hydrophobicity.

Materials and Methods

Cellular material

The following microorganisms were studied. Bacteria: *Acetobacter aceti* ATCC-23747, ATCC-23748 and subsp. *xylinum* NCIB-8246. *Enterobacter aerogenes MBLA-0400; Klebsiella oxytoca* NRRL-13199; *Bacillus licheniformis* DSM-603 *Corynebacterium glutamicum* ATCC-13032. Yeast-like fungus: *Moniliella pollinis* MUCL-11525. Yeasts: *Kluyveromyces fragilis* MUCL-2774; *Saccharomyces carlsbergensis* M-259; *Saccharomyces cerevisiae* D273-10B.

Culture and harvest conditions were described previously [16,18], except for the following. *S. cerevisiae* was cultivated in a medium composed of 50 g/l glucose and 20 g/l yeast extract (Difco); *B. licheniformis* was cultivated in agitated erlenmeyer flasks at 37 °C in media containing 10 g/l peptone, 5 g/l glucose and 5 g/l NaCl during 17 h, then in a media composed of 10 g/l starch, 10 g/l peptone, 5 g/l meat extract, 2.5 g/l NaCl and $1 \cdot 10^{-5}$ mol/l $MnSO_4$ during 24 h, unless specified otherwise; *C. glutamicum* was cultivated in turbidostat as described by Buchs et al. [19]. After the harvest, cells were washed three times and suspended in distilled water unless otherwise specified. The cellular concentration of this suspension was determined by direct count and by turbidimetry (at 600 nm).

Analytical methods

(1) The elemental surface composition of the cells was determined by XPS analysis; the procedure used is as described by Amory et al. [10]. The pellet of the last washing after harvest was frozen in liquid nitrogen and dried under vacuum at −5 °C (Lyovac GT4, Leybold Heraeus). The freeze-dried cell powder was pressed (3 T/cm^2) in a stainless steel trough of 8 mm diameter in order to obtain smooth surface. XPS analysis was performed at room temperature with a Vacuum Generators ESCA 3 Mk II spectrometer equipped with a Tractor Northern TN 1710 signal averager. The residual pressure in the spectrometer was in the range of $(1–5) \cdot 10^{-9}$ Torr. A Mg anode (1253.6 eV) with an Al window was used for X-ray production; it was powered at 14 kV and the beam current was 20 mA. The constant pass energy in the hemispherical analyser of 50 eV and the slit width of 4 mm gave an energy resolution of 1 eV for detailed spectra. The angle between the sample surface and the direction of electrons detection was 45°. The recording sequence and duration of

each accumulation (in min) were the following: general spectrum with a pass energy of 100 eV, C_{1s} (5), O_{1s} (5), N_{1s} (35), P_{2p} (45), C_{1s} (5). The energy scale of the spectrometer was calibrated with $Au_{4f7/2}$ at 84.0 eV binding energy; charge correction was made by fixing the C_{1s} binding energy of carbon not bound to oxygen at 285 eV. The intensities were estimated by calculating the integral of each peak with the internal routine of the signal averager after smoothing and linear background subtraction. The atomic concentration ratios were calculated by correcting the intensity ratios with experimental sensitivity factors computed by Wagner et al. [20].

(2) The surface charge of the microorganisms was characterized by determining their electrophoretic mobility with a Pen Kem Laser-Zee-Meter model 500. Freshly harvested cells were suspended in distilled water, at a concentration of $2 \cdot 10^{10}$ cell/l for bacteria and $2 \cdot 10^{9}$ cell/l for yeasts. The mobility was measured as a function of pH: for each measurement a distinct portion of the suspension was taken and its pH was adjusted by addition of HNO_3 or NaOH just before the measurement. The ionic strength was not adjusted but was kept as low as possible by avoiding any addition of electrolyte. This practice was adopted in order to allow extension of the diffuse double layer and as a consequence to enhance the absolute value of the electrophoretic mobility and the differences between the various species.

(3) The hydrophobicity of the cells was evaluated by two methods: contact angle measurements (a) and hydrophobic interaction chromatography (HIC) (b).

(a) Contact angles of water were determined on cellular films prepared from suspensions of freshly harvested cells as described previously [16]. Sessile drops of the advancing type were measured by use of a home-made instrument. It is an optical-bench type device, incorporating an illumination source, a stage on which the sample is placed, a microsyringe for drop formation, a short distance telescope and a projection screen. The enlarged image of the drop is observed as a silhouette; its height and base width are determined and the contact angle is calculated.

(b) The retention of cells by a hydrophobic gel was measured under four different conditions:

H3, high ionic strength (4 M NaCl) at pH 3 (fixed with HCl).
L3, low ionic strength (no salt added), pH 3.
H7, high ionic strength (4 M NaCl), pH 7 (fixed with NaOH).
L7, low ionic strength (no salt added), pH 7.

The column was a short ended Pasteur pipette, plugged with glass-wool; the gel was Phenyl Sepharose CL-4B (Pharmacia); it was packed in the column to a height of 3 cm and equilibrated with one of the solutions described above (H3, L3, H7 or L7). A portion of the original cellular suspension was centrifuged and the cells were resuspended in the desired solution to give a suspension with optical density of about 18 at 600 nm; 0.1 ml of that suspension was applied to the column and followed by 3 ml of the same solution. The eluent was collected and the proportion of retained cells (R) was calculated by the equation:

$$R = 100(A_i - A_o)/A_i$$

where A_i is the absorbance of 0.1 ml of the cellular suspension diluted with 3 ml of water, and A_o is the absorbance of the eluent.

Results

Elemental composition

The XPS analysis was performed on samples from two independent cultures for each one of the microorganisms. The average values, expressed as atomic concentration ratios, are given in Table I. Carbon is the most abundant element, as expected. The concentration ratio of oxygen to carbon varies between 0.21 to 0.53. Nitrogen is found in lower concentrations; the N/C ratio of the fungi (0.013–0.024) is clearly lower than that of the bacteria (0.040–0.082). Phosphorus is detected at still lower concentrations and, like nitrogen, is more abundant at the surface of the bacteria as compared to the fungi.

Millard et al. [8] analysed the surface composition of some Gram-positive and Gram-negative bacteria. Our results agree with their data in revealing that different species show a larger variation of the phosphorus level compared to other elements, and in pointing *Bacillus* species as hav-

TABLE I

SURFACE CHEMICAL COMPOSITION OF DIFFERENT MICROORGANISMS

Microorganism	Atomic ratios			
	O/C	N/C	P/C	N/P
A. aceti ATCC 23747	0.40	0.055	0.0029	19.1
A. aceti ATCC 23748	0.39	0.059	0.0031	19.2
A. aceti NBIC 8246	0.23	0.064	0.0059	10.8
E. aerogenes	0.49	0.044	0.0051	8.4
K. oxytoca	0.52	0.040	0.0025	16.0
B. licheniformis	0.29	0.082	0.0221	3.7
C. glutamicum	0.21	0.040	0.0047	8.5
M. pollinis	0.28	0.018	0.0012	15.9
K. fragilis	0.53	0.024	0.0026	9.0
S. carlsbergensis	0.31	0.013	0.0013	10.0
S. cerevisiae	0.30	0.014	0.0022	6.4

ing the strongest phosphorus signal. Van Haecht et al. [9] also showed highest variability of the phosphorus surface concentrations when comparing different yeast species and culture conditions. Buchs et al. [19] detected very low concentrations in *C. glutamicum* cultivated in phosphate-depleted medium as compared to unlimiting medium.

In a set of experiments with *B. licheniformis*, the bacteria were cultivated under two different aeration regimes by using baffled erlenmeyer flasks or normal ones. Cells were harvested at different moments of the culture and submitted to XPS analysis. A marked decrease of the phosphorus concentration was observed for bacteria grown under the more efficient aeration (in baffled flasks) as a function of culture age.

Fig. 1 shows some representative C_{1s} and O_{1s} peaks. The shape of the C_{1s} peak varies appreciably from one microorganism to another. The peak has been decomposed into three components, assuming a pure Gaussian shape and keeping a constant full width at half maximum of 2.15 eV, established in the analysis of yeasts [10]; the binding energy and the intensity of the components were determined by a best fitting program. The components were identified according to Ratner and McElroy [20]: the component at 285.0 eV is due to carbon involved only in C-C and C-H bonds (hydrocarbon), that at 286.6 ± 0.1 eV is due to C-O and C-N moieties (ether, alcohol and amine), and that at 288.4 ± 0.1 eV is attributed to C = O and HNC = O (ketone, amide).

The binding energy scale was adjusted according to the position of the low energy C_{1s} component set at 285.0 eV; positions of all the other peaks were determined accordingly.

Carboxyl groups are expected to give a component at 289.1 eV. Their contribution is thus included in the right-hand side of the peak assigned to ketone and amide, but represents only a minor proportion of its intensity. Using a fourth component to account specifically for carboxyl would be justified in principle; however, it would not give reliable information. In fact, the accuracy of the data related to the small peak assigned to ketone and amide is already rather poor because of the crucial dependence of the assumption of Gaussian shape [13].

The O_{1s} peak of the yeast appears at a binding energy of 533.2 ± 0.1 eV and is symmetrical. Asymmetry is observed in the shape of the O_{1s} peak of the bacteria. Decomposition of this peak into two components was done as described above, taking a full width at half maximum of 2.6 eV which is the mean value of all the symmetrical O_{1s}

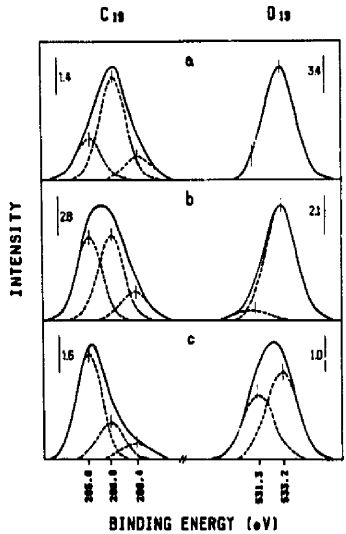

Fig. 1. Representative XPS peaks, C_{1s} and O_{1s}: a, *Kluyveromyces fragilis*; b, *Acetobacter aceti* ATCC 23747; c, *Bacillus licheniformis*. The scale bar allows a comparison in terms of normalized intensity.

TABLE II

SURFACE CONCENTRATIONS, RELATIVE TO TOTAL CARBON, OF THE DIFFERENT FORMS OF CARBON AND OXYGEN

Microorganism	Carbon			Oxygen	
	(C-H)/C	(C-O)/C or (C-N)/C	(C=O)/C	(X-OH)/C	(X = O)/C or (X-O-)/C
A. aceti ATCC 23747	0.45	0.41	0.14	0.36	0.04
A. aceti ATCC 23748	0.47	0.40	0.13	0.36	0.03
A. aceti NBIC 8246	0.62	0.28	0.10	0.18	0.05
E. aerogenes	0.39	0.47	0.14	0.44	0.05
K. oxytoca	0.34	0.51	0.15	0.47	0.05
B. licheniformis	0.62	0.27	0.11	0.18	0.11
C. glutamicum	0.70	0.21	0.09	0.15	0.06
M. pollinis	0.58	0.32	0.10	0.28	0
K. fragilis	0.31	0.56	0.13	0.53	0
S. carlsbergensis	0.57	0.35	0.08	0.31	0
S. cerevisiae	0.60	0.33	0.07	0.30	0

peaks in this study. The component at 533.2 ± 0.1 eV is attributed to oxygen engaged in hydroxide group; the component at 531.3 ± 0.1 eV is assigned to oxygen bound otherwise (to carbon, to phosphorus etc.). These positions should be taken as first approximation only; exact values in published literature do not always agree, but hydroxide oxygen is generally reported to have binding energy 1–2 eV higher than other types of oxygen [21–25].

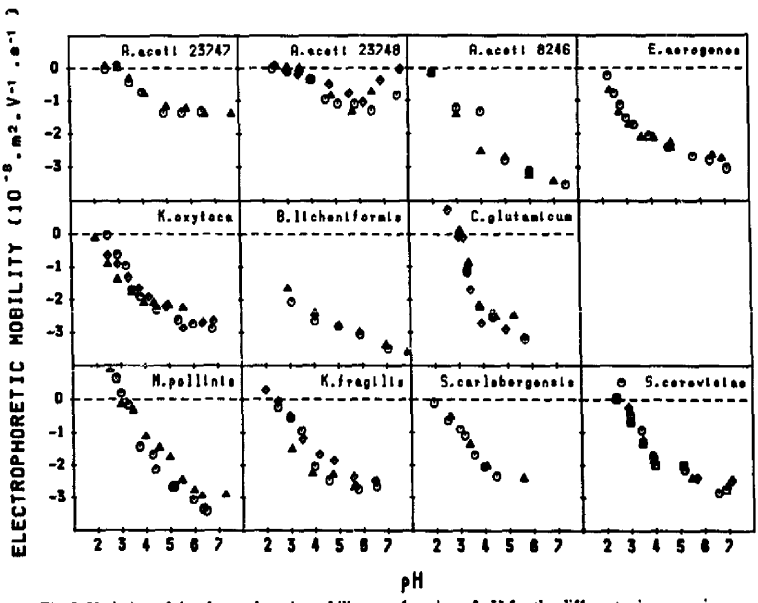

Fig. 2. Variation of the electrophoretic mobility as a function of pH for the different microorganisms.

TABLE III

PHYSICO-CHEMICAL PROPERTIES OF DIFFERENT MICROORGANISMS; CONFIDENCE INTERVAL AT 99% LEVEL IS GIVEN BETWEEN BRACKETS

Microorganism	Hydrophobicity					Charge (IEP)
	contact angle (°)	retention by Phenyl-Sepharose (%)				
		H3	L3	H7	L7	
A. aceti ATCC 23747	n.d.	n.d.	n.d.	n.d.	n.d.	3.1
A. aceti ATCC 23748	19 (0)	41 (4)	12 (5)	38 (4)	3 (1)	3.0
A. aceti NBIC 8246	n.d.	n.d.	n.d.	n.d.	n.d.	*1.9
E. aerogenes	43 (1)	49 (1)	25 (7)	44 (1)	3 (2)	*2.0
K. oxytoca	29 (1)	44 (2)	18 (2)	37 (3)	6 (2)	*2.2
B. licheniformis	53 (2)	83 (3)	51 (7)	70 (2)	24 (5)	*2.1
G. glutamicum	49 (2)	95	96	91	0	*2.9
M. pollinis	115 (1)	100 (0)	100 (0)	100 (0)	54 (10)	3.0
K. fragilis	32 (1)	100 (0)	98 (1)	99 (1)	4 (1)	2.4
S. carlsbergensis	53 (2)	96 (1)	42 (6)	75 (6)	7 (2)	2.0
S. cerevisiae	16 (0)	32 (4)	38 (9)	22 (3)	6 (2)	2.4

n.d., not determined.

* Approximate value, obtained by extrapolation.

Table II presents the concentrations of the different forms of carbon and oxygen relative to the total carbon concentration.

The small N_{1s} peak at 400.3 ± 0.1 eV is rather noisy; therefore its shape and symmetry cannot be judged. In principle, amide (binding energy 400.3 eV) cannot be distinguished from amine (400.1 eV) [21]. However, protonated amine (401.9 eV) should be recognised.

The P_{2p} peak appears at binding energy of 133.7 ± 0.2 eV which is characteristic of phosphate [24].

Physico-chemical properties

The electrophoretic mobilities of samples from two or three different cultures of each microorganism were determined as a function of pH. The results are presented in Fig. 2 and the isoelectric points are included in Table III.

The curves obtained for most of the microorganisms are similar in trend, but differ in steepness. *A. aceti* ATCC 23748 is distinguished from all the other species by having a curve with a minimum around pH 5–6. Amory and Rouxhet [12] have observed that the electrophoretic mobility curve of yeast has a minimum at pH 5. This was attributed to an increase of ionic strength resulting from the addition of NaOH in order to raise the pH above 5. Such a minimum was not observed for yeasts in the present work due to the shorter time (a few minutes instead of 1 h) between pH adjustment and the electrophoretic mobility measurement. The observation for *A. aceti* ATCC 23748 may be due to a faster release of protons or other ions as compared to the other strains, resulting in an increase of the ionic strength.

Contact angles of water were determined on samples emerging from two or three independent cultures of each microorganism. HIC was run in duplicate on fresh cells from 2–4 independent cultures suspended in the four solutions described above. Table III summarizes the hydrophobicity parameters: contact angle and retention by hydrophobic gel at different pH and ionic strength conditions.

Discussion

Functional composition

Fig. 3 presents the correlation between the relative intensity of the components of the C_{1s} peak which are due to carbon bound to oxygen and nitrogen (286.6 and 288.4 eV), on the one hand,

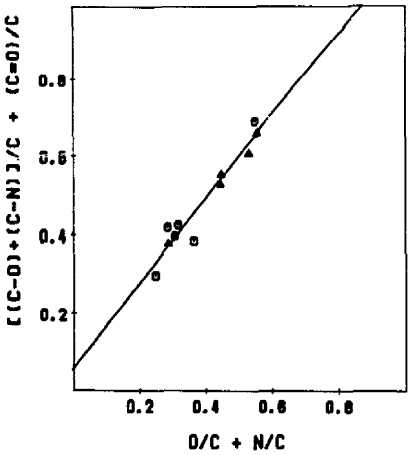

Fig. 3. Proportion of the C_{1s} peak components due to carbon bound to oxygen and nitrogen as a function of the sum of oxygen/carbon and nitrogen/carbon concentration ratios: △, Gram-negative bacteria; □, Gram positive bacteria; ○, yeasts. The correlation coefficient is 0.98.

and the sum of the O/C and N/C atomic concentration ratios, on the other hand. The good correlation, the small value of the intercept and the fact that the slope is close to unity, confirm the validity of the sensitivity factors and the overall consistency of the C_{1s} peak decomposition.

In the Gram-negative bacteria, except *A. aceti* 8246, a high proportion of the carbon peak (40–50%) is due to C-O (or C-N); the O/C ratio is relatively high (0.4–0.5) and most of the oxygen (91%) is engaged in hydroxide functions (probably as C-OH and P-OH). These observations are in agreement with the presence of lipopolysaccharides in the outer membrane. About 14% of the carbon is in C=O functions; the rather low N/C ratio shows that at most one third of this portion can be in amide.

The two Gram-positive bacteria, *B. licheniformis* and *C. glutamicum*, are characterized by the lowest O/C ratio. Analysis of the components of the O_{1s} peak shows that the concentration of hydroxide oxygen is 2–3-times lower in these bacteria as compared to the Gram-negative bacteria, although the concentrations of oxygen participating in ketone or amide are similar in both groups. This indicates that polysaccharides constitute only a small proportion of the surface of Gram-positive bacteria. Decomposition of the C_{1s} peak reveals that they have the highest proportion of hydrocarbon type carbon, which represents probably the apolar moieties of proteins and the lipidic moieties of lipoteichoic acids found on the external side of their cell wall; Baddiley et al. [7] reported a similar observation for *B. licheniformis*.

A. aceti NCIB 8246 exhibits characteristics which are similar to the Gram-positive bacteria: low hydroxide contribution in the oxygen peak and high hydrocarbon contribution in the carbon peak. These are rather surprising observations for a bacterium which is known to have a cellulosic capsule.

In the yeasts, all the oxygen seems to be in hydroxide form. Taking *K. fragilis* aside, the O/C ratio is about 0.3, which is much lower than what can be expected for carbohydrates; the high contribution of C-H in the C_{1s} peak suggests a presence of proteins or lipids. *K. fragilis* is a unique case, showing a very high O/C ratio concomitantly with an extremely high proportion of oxygen bound carbon.

Chemical composition and surface charge

Fig. 4 presents the correlation between the electrophoretic mobility at pH 4 and the N/P con-

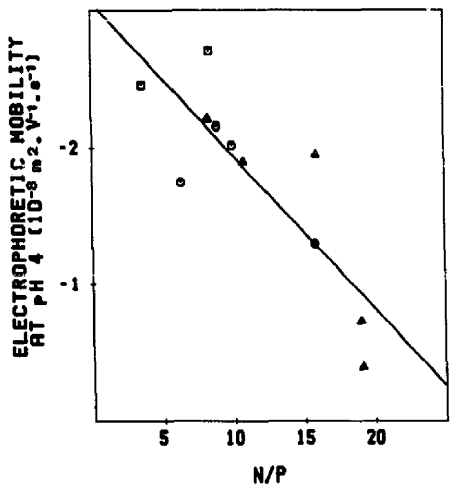

Fig. 4. Variation of the electrophoretic mobility at pH 4 as a function of nitrogen to phosphorus concentration ratio: △, Gram-negative bacteria; □, Gram-positive bactria; ○, yeasts. The correlation coefficient is 0.83.

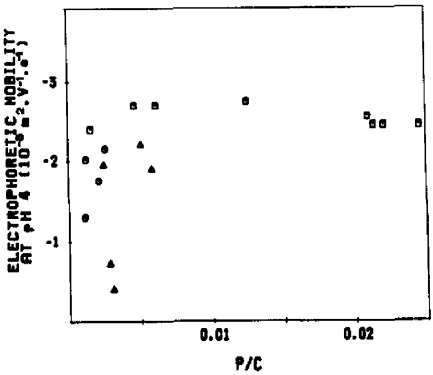

Fig. 5. Variation of the electrophoretic mobility at pH 4 as a function of phosphorus to carbon ratio: △, Gram-negative bacteria; □, Gram-positive bacteria including data concerning cells cultivated under different aeration or nutrition conditions, or harvested at different ages; ○, yeasts.

centration ratio. Similar observations were reported recently [10,11]. Such trends were observed at any pH with varying degrees of correlation. This suggests that the negative surface charge due to deprotonation of phosphates is partially neutralized by protonation of amines (in basic amino acids, in phospholipids, etc.) which constitute only a fraction of the total nitrogen on the surface of the cell.

Fig. 5 shows the variation of electrophoretic mobility at pH 4 as a function of P/C concentration ratio. It includes data obtained for *C. glutamicum* cultivated under different nutritive regimes [19] and for *B. licheniformis* grown under varying aeration conditions and harvested at varying ages. The mobility increases with phosphorus concentration up to a P/C ratio of about 0.006, then it remains practically constant. Similar profiles were obtained at all the pH investigated, the level of the plateau varying with the pH. The contribution of phosphate or phosphoester (pK_a between 1 and 2), to charge at the pH range under study is straightforward.

Phosphomannans are an important constituent of the cell wall of yeast [6]; the presence of polyphosphate outside the plasma membrane of yeast cells has also been noted [26]. Jayatissa and Rose [27] have demonstrated the predominant role of phosphate in the surface charge of yeast by showing that removing the phosphorus from the wall renders the cells almost neutral.

Phosphate groups are constituents of most amphiphile molecules in bacterial wall: lipoteichoic acids in Gram-positive bacteria, phospholipids and lipopolysaccharides in Gram-negative bacteria.

Leveling of the zeta potential as the phosphorus concentration increases beyond a certain value has also been observed for a collection of yeasts [13]. It may be attributed to two effects occurring as the potential and the surface density of charge become more negative: the apparent pK_a of the remaining acidic groups increases [28] and counterion condensation becomes more important [29]. A surface potential of $-E$ mV is expected to induce an increase of $E/25.7$ of the apparent pK for the dissociation of an acid function or of a surface complex formed between a negative site and a monovalent cation.

Van der Mei et al. studied recently various strains of *Streptococcus* [30] and mutants of *Streptococcus salivarius* [31]. They reported an increase of isoelectric point accompanying an increase of surface nitrogen concentration (measured by XPS) and increased exposure of proteins on the surface (determined by immunological tests). Our data do not give any correlation between the electrophoretic mobility and the N/C ratio. It appears thus that the contribution of nitrogen in the correlation with the N/P ratio should be considered with caution. Nitrogen's direct influence may be significant only when closely related microorganisms are compared and does not appear when a broad collection of cells is studied.

It is generally accepted that carboxyl groups play a role in determining the negative charges on the cell surface; the measurements reported here do not provide direct information on this subject.

A correlation has been reported between the electrophoretic mobility and the phosphate surface concentration of different yeast strains in the low concentration range [12]; this indicates that phosphates play a predominant role in the development of the surface charge and that the participation of carboxyl groups is minor. Fig. 5 shows that in the present work, which involves both yeast and bacteria, the correlation obtained at low P/C ratios is much looser.

As pointed out above, carboxyls represent only

a small proportion of the carbon in C=O functions and of the oxygen in OH functions. However, comparison of Tables I and II shows that a carboxyl concentration higher than the phosphate concentration is still possible. A contribution of carboxyl in determining the surface charge may not be excluded. However, it must be kept in mind that presence of carboxyl groups on the surface does not necessarily imply that they contribute to the surface charge. It has been mentioned above that at high phosphorus concentrations the negative potential generated by already dissociated phosphate groups causes an increase of the apparent pK_a of phosphate groups which are still protonated. By the same reasoning the ionization of carboxyl groups is expected to be weak due to the influence of the neighbouring phosphate groups. Such an effect was described by James et al. [28] for dissociation of carboxyl groups next to sulfate groups.

Hydrophobicity

A good agreement is obtained between the HIC retentions at pH 3 and pH 7 when a high ionic strength is used (H3 and H7). It may thus be considered that the cell surface charge is efficiently screened and does not influence the hydrophobicity determination. When HIC is performed at pH 3 and low ionic strength (L3), comparison between the cell species is not done on the same basis in terms of electrostatic interactions, since at that pH some species are at their isoelectric point while the others are above their isoelectric point. Therefore these data should not be taken into consideration. Except for two strains of *A. aceti*, the electrophoretic mobilities at pH 7 of the different cells are rather similar. Under condition L7 the electrostatic interactions and the high water activity do not favour retention by the hydrophobic gel; HIC retention is indeed very low except for *M. pollinis*. This points to the very hydrophobic character of the latter, also indicated by the high water contact angle, and allows to distinguish it from *K. fragilis*, while their HIC retention at high ionic strength is the same.

Therefore a HIC index has been calculated as the average of the retentions measured in the conditions H3, H7 and L7. The correlation coefficient between the HIC index and the water contact angle is +0.80. The retention parameter from HIC, representing hydrophobicity nuanced by electrostatic interactions, agrees only roughly with

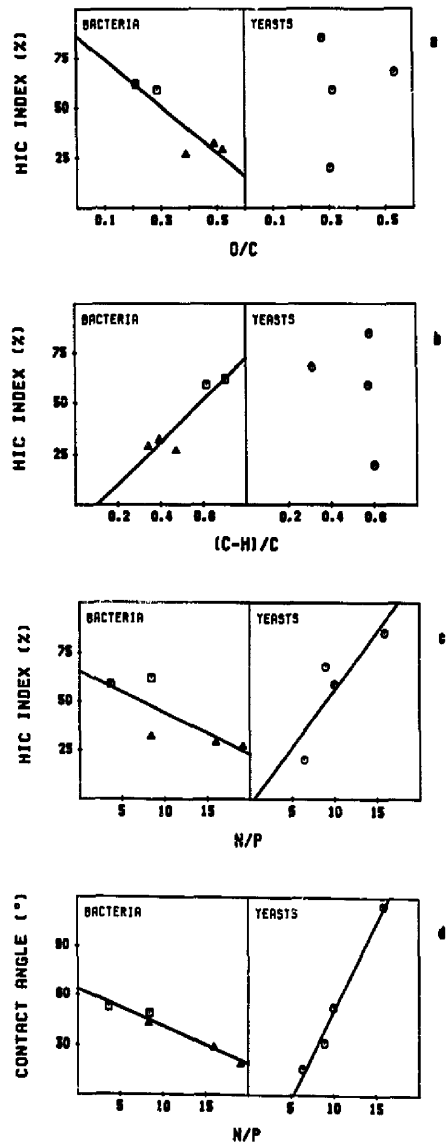

Fig. 6. Variation of hydrophobicity parameters (HIC index, water contact angle) as a function of surface composition: (a) atomic ratio O/C; (b) proportion of carbon not bound to oxygen or nitrogen, (C-H)/C; (c, d) atomic ratio N/P. △, Gram-negative bacteria; □, Gram-positive bacteria; ○, yeasts.

the hydrophobicity parameter that represents pure surface energy (contact angle).

Chemical composition and hydrophobicity

The tendency to avoid water results from the presence of non-polar and non-polarizable regions on the surface. The presence of oxygen or nitrogen favours interactions with water molecules (via H-bonds) and should reduce hydrophobicity. Indeed, Fig. 6 shows that hydrophobicity of bacteria is inversely correlated with the O/C ratio reflecting polar functions. It is directly correlated with the relative intensity of the hydrocarbon component of the C_{1s} peak, which is due to apolar moieties in proteins, lipoproteins, phospholipids or lipopolysaccharides. However, the yeasts do not show similar correlations; a large variation is observed between the hydrophobicities of three of the strains while their O/C and (C-H)/C ratios are practically constant.

Fig. 6 shows also that the N/P ratio provides a further distinction between bacteria and yeasts. The former are more hydrophilic while the latter are more hydrophobic as N/P increases. A positive correlation between N/P ratio and hydrophobicity of yeasts had already been described [12]. The role of proteins in determining yeast hydrophobicity was illustrated by use of proteolytic treatment [32]. The difference shown between bacteria and yeasts might be due to a difference of composition or configuration of the proteins; another explanation could be that proteins account for most of the surface nitrogen in the case of yeasts and only for a small part in the case of bacteria.

Conclusions

This work presents a preliminary attempt to exploit a combination of different techniques of surface characterization for the investigation of microorganisms. It is evident that a small collection of broad taxonomic variety could not provide results of definite statistical significance. However, the approach seems to be valuable.

Polysaccharides constitute a large proportion of the surface of any microorganism. That proportion varies from one species to the other. Generally, it is high in Gram-negative bacteria, lower in yeasts and still lower in Gram-positive bacteria. The presence of macromolecules rich in hydrocarbon moieties (proteins, lipids, lipoproteins, lipopolysaccharides, etc.) is indicated in Gram-positive bacteria and in yeasts.

Overall physico-chemical properties are strongly dependent on the ratio of nitrogen to phosphorus. Phosphate clearly contributes to enhanced negative character and nitrogeneous functions can be linked to decrease of the negative charge; the ratio of nitrogen to phosphorus is a good estimator of the cellular surface charge.

The influence of the concentrations of oxygen, nitrogen and hydrocarbon moieties on the hydrophobicity of bacteria follows the trend expected from the polarity of corresponding functions. The situation is different for yeasts, the hydrophobicity of which is related only to the N/P ratio, reflecting the abundance of proteins.

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References

1 Wicken, A.J. (1985) in Bacterial Adhesion (Savage, D.C. and Fletcher, M., eds.), pp. 45–70, Plenum Press, New York and London.
2 Shockman, G.D. and Barrett, J.F. (1983) Annu. Rev. Microbiol. 37, 501–527.
3 Costerton, J.W., Ingram, J.M. and Cheng, K.J. (1974) Bacteriol. Rev. 38, 87–110.
4 Sleytr, U.B. (1978) Int. Rev. Cytol. 53, 1–74.
5 Aronson, J.M. (1981) in Biology of Conidial Fungi (Cole, G.T. and Kendrick, B., eds.), Vol. 2, pp. 459–507, Academic Press, London.
6 Bacon, J.S.D. (1981) in Yeast Cell Envelope Biochemistry, Biophysics and Ultrastructure (Arnold, W.N., ed.), Vol. 1, pp. 65–84, CRC Press, Boca Raton, FL.
7 Baddiley, J., Hancock, I.C. and Sherwood, P.M.A. (1973) Nature 243, 43–45.
8 Millard, M.M., Sherrer, R. and Thomas, R.S. (1976) Biochem. Biophys. Res. Commun. 72, 1209–1217.
9 Van Haecht, J.L., Defosse, C., Van den Bogaert, B. and Rouxhet, P.G. (1982) Coll. Surfaces 4, 343–358.

10 Amory, D.E., Genet, M.J. and Rouxhet, P.G. (1988) Surf. Interface Anal. 11, 478–486.
11 Amory, D.E., Mozes, N., Hermesse, M.P., Leonard, A.J. and Rouxhet, P.G. (1988) FEMS Lett. 49, 107–110.
12 Amory, D.E. and Rouxhet, P.G. (1988) Biochim. Biophys. Acta 938, 61–70.
13 Amory, D.E., Dufour, J.P. and Rouxhet, P.G. (1988) J. Inst. Brew. 94, 79–84.
14 Rosenberg, M. and Kjelleberg, S. (1986) in Advances in Microbial Ecology (Marshall, K.C., ed.), Vol. 9, pp. 353–393, Plenum Publishers, New York.
15 Dillon, J.K., Fuerst, J.A., Haywards, A.C. and Davis, G.H.G. (1986) J. Microbiol. Methods 6, 13–19.
16 Mozes, N. and Rouxhet, P.G. (1987) J. Microbiol. Methods 6, 99–112.
17 Van der Mei, H.C., Werkamp, A.H. and Busscher, H.J. (1987) J. Microbiol. Methods 6, 277–287.
18 Mozes, N., Marchal, F., Hermesse, M.P., Van Haecht, J.L., Reuliaux, L., Leonard, A.J. and Rouxhet, P.G. (1987) Biotechnol. Bioeng. 30, 439–450.
19 Buchs, J., Mozes, N., Wandrey, C. and Rouxhet, P.G. (1988) Appl. Microbiol. Biotechnol., in press.
20 Wagner, C.D., Davis, L.E., Zeller, M.V., Taylor, J.A., Raymond, R.H. and Gale, L.M. (1981) Surf. Interface Anal. 3, 211–225.
21 Ratner, B.D. and McElroy (1985) in Spectroscopy in the Biomedical Sciences (Gendreau, R.M., ed.), pp. 108–140, CRC Press, Boca Raton, FL.
22 Clark, D.T., Peeling, J. and Colling, L. (1976) Biochim. Biophys. Acta 453, 533–545.
23 Cothern, R.C., Moddeman, W.E., Albidge, R.G., Sandress, W.J., Kelly, P.L., Hanley, W.S. and Field, L. (1976) Anal. Chem. 48, 162–166.
24 Landis, W.J. and Martin, J.R. (1984) J. Vac. Sci. Technol. A2, 1108–1111.
25 Dilks, A. (1981) ACS Symp. 162 (Photon, Electron, Ion Probes Polym. Struc. Prop.), 293–317.
26 Tijssen, J.P.F., Van Steveninck, J. and De Bruijn, W.C. (1985) Protoplasma 125, 124–128.
27 Jayatissa, P.M. and Rose, A.H. (1976) J. Gen. Microbiol. 96, 165–174.
28 James, R.O., Davis, J.A. and Leckie, J.O. (1978) J. Coll. Interf. Sci. 65, 331–344.
29 Lyklema, J. (1985) in Surface and Interface Aspect of Biomedical Polymers (Andrade, J.D., ed.), Vol. I, pp. 293–336, Plenum Press, New York and London.
30 Van der Mei, H.C., Leonard, A.J., Weerkamp, A.H., Rouxhet, P.G. and Busscher, H.J. (1988) Colloids Surfaces 32, 297–305.
31 Van der Mei, H.C., Leonard, A.J., Weerkamp, A.H., Rouxhet, P.G. and Busscher, H.J. (1988) J. Bacteriol. 170, 2462–2466.
32 Kadama, K. and Murata, M. (1984) Agr. Biol. Chem. 48, 2423–2433.