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The Relation Between the Surface Chemical Composition of *Escherichia coli* and their Electron Donor/Electron Acceptor (Acid-base) Properties

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ABSTRACT

The physicochemical properties (hydrophobicity, electron donor/electron acceptor properties (acid-base) of three *E. coli* strains have been investigated using contact angle measurements. Two conditions of culture were used: The cells were grown in Liquid Luria Bertani medium (LLB) and in Solid Luria Bertani medium (SLB). Based on free energy of interactions (ΔG^{iwi}), we found that all *E. coli* strains have hydrophilic character. Moreover, the cell surface of *E. coli* strains is predominately electron donor and weakly electron acceptor. The relationships between electron donor/electron acceptor (acid-base) properties and surface chemical composition determined by X-ray Photoelectron Spectroscopy (XPS) were investigated. We show that the phosphate groups play the crucial role in determining the electron donor (base) property and the amine groups were responsible for decreasing the electron acceptor (acid) property. The relation between surface molecular compositions determined by modeling the XPS data and the electron donor/electron acceptors properties were also examined. High concentration of polysaccharides seems to be responsible for high electron acceptor (acid) property. Moreover, the ratio of polysaccharides/proteins could be an origin of the electron acceptor property of studied cells surface.

Key words: *Escherichia coli*, surface chemical composition, surface molecular composition, acid base properties, contact angle measurements

INTRODUCTION

Physicochemical surface characteristics of both cell surface and solid surface determine the attractive and the repulsive forces between these surfaces and therefore play a crucial role in adhesion and biofilm formation. These characteristics comprise surface charge, hydrophobicity and electron donor-electron acceptor properties (acid-base). A better knowledge of origin of all physicochemical properties in term of chemical composition of the cell surface is required in order to understand these properties and therefore fully understand the adhesion process. The surface

chemical composition and the molecular surface composition have been related to cell surface hydrophobicity assessed by water contact angles (Amory *et al.*, 1988; Boonaert and Rouxhet, 2000; Cowan *et al.*, 1992; Cuperus *et al.*, 1993; Dufrene and Rouxhet, 1996a; Latrache *et al.*, 2002; Mozes *et al.*, 1988; Mozes *et al.*, 1989; Van der Mei and Busscher, 1997). These studies revealed that the hydrophobic character is related to concentration of nitrogen or carbon in hydrocarbon form, and to concentration of proteins whereas a hydrophilic surface was associated with low concentration of hydrocarbon, high concentration of oxygen and with the presence of polysaccharides.

The relation between surface charge and surface chemical composition has been also studied (Amory *et al.*, 1988; Boonaert and Rouxhet, 2000; Cowan *et al.*, 1992; Dengis and Rouxhet, 1997; Fatima *et al.*, 2005; Latrache *et al.*, 1994; Mozes *et al.*, 1988; Mozes *et al.*, 1989; Van der Mei *et al.*, 1989; Van der Mei and Busscher, 1997). Some of these works (Amory *et al.*, 1988; Cowan *et al.*, 1992; Latrache *et al.*, 1994; Mozes *et al.*, 1988; Mozes *et al.*, 1989; Van der Mei *et al.*, 1989; Van der Mei and Busscher, 1997) have shown that the phosphate groups play the major role in determining the surface charge and the higher isoelectric points is related to higher N/C surface concentration. The works based on modeling surface charge according to the surface chemical composition given by X-ray photoelectron spectroscopy (Boonaert and Rouxhet, 2000; Dengis and Rouxhet, 1997; Fatima *et al.*, 2005; Hamadi *et al.*, 2008) have reported that also carboxylic groups contribute in determining the surface charge. As reported previously, the relation between chemical surface composition, molecular surface composition and surface charge or/and hydrophobicity have been studied extensively. In contrast, attempts to relate the acid-base properties (electron donor-electron acceptor) and chemical surface composition, molecular surface composition are scarce (Sharma and Hanumantha, 2002) despite the crucial role of the acid-base interactions in adhesion phenomenon and their high importance than the other interactions (Van Oss, 1993).

In the present study the relationships between surface chemical composition, modeling molecular surface composition and acid-base properties (contact angle measurements) are examined for *E. coli*.

MATERIALS AND METHODS

Bacterial strains and growth conditions: Three *E. coli* strains used are: (i) HB101, a K12 strain, non-pathogenic; (ii) 382 and (iii) AL52, both isolated from patients with urinary tract infection. Each strain was grown either in Liquid Luria Bertani medium (LLB) or on Solid Luria Bertani agar (SLB) overnight at 37°C. The culture was harvested by centrifugation for 15 min at 8400 g, washed twice with KNO_3 solution and resuspended in for contact angle measurement.

Contact angle measurements: The method for measuring contact angles on bacterial layers has been described (Busscher *et al.*, 1984).

The Lifshitz-Van der Waals (γ^{LW}), electron donor (γ^-) and electron acceptor (γ^+) components of the surface tension of bacteria (B) were estimated from the approach proposed by Van Oss *et al.* (1988a). In this approach the pure liquid (L) contact angles (θ) can be expressed as:

$$\text{Cos}\,\theta = -1+2(\gamma_B^{LW}\gamma_L^{LW})^{1/2}/\gamma_L+2(\gamma_B^{+}\gamma_L^{-})^{1/2}/\gamma_L+2(\gamma_B^{-}\gamma_L^{+})^{1/2}/\gamma_L.$$

The Lewis acid-base surface tension component is defined by:

$$\gamma_B^{AB} = 2(\gamma_B^- \gamma_B^+)^{1/2}$$

The cell surface hydrophobicity was evaluated through contact angle measurements and by the approach of Van Oss *et al.* (1988a) and Van Oss (1995). In this approach, the degree of hydrophobicity of a given material (*i*) is expressed as the free energy of interaction between two entities of that material when immersed in water (w): ΔGiwi. If the interaction between the two entities is stronger than the interaction of each entity with water, the material is considered hydrophobic (ΔGiwi<0); conversely, for a hydrophilic material, ΔGiw*i*>0. ΔGiwi is calculated through the surface tension components of the interacting entities, according to the following formula:

$$\Delta G_{iwi} = -2\gamma_{iw} = -2[((\gamma_i^{LW})^{1/2} - (\gamma_w^{LW})^{1/2})^2 + 2((\gamma_i^+ \gamma_i^-)^{1/2} + (\gamma_w^+ \gamma_w^-)^{1/2} - (\gamma_i^+ \gamma_w^-)^{1/2} - (\gamma_w^+ \gamma_i^-)^{1/2})]$$

X-ray photoelectron spectroscopy analysis (XPS): Bacteria were collected by centrifugation suspended in distilled water and washed twice. The pellet was kept frozen at-80°C. Samples were lyophilized in a Lyovac GT4 (Leyobold Heraeus). The elemental composition of the cell surface was determined by XPS using a SSX 100 Spectrometer (Model 206, Surface Science Instruments) with A1-anode for X-ray production; experimental details were described previously (Latrache *et al.*, 1994).

The functional composition was obtained by the decomposition of XPS peaks, using least-squares curves fitting. The carbon peak was decomposed into three components set at 284.8, 286.2, 287.9 eV representing carbon singly bound to carbon and hydrogen (C-(C,H), carbon singly bound to oxygen or nitrogen (C-(O, N) and carbon making a double bond or two single bonds with oxygen (C = O), respectively. The oxygen peak was decomposed into two components set at 531.2, 532.6 eV representing oxygen making a double bond with carbon (O = C) and oxygen involved in hydroxide or ether functions (OH, C-O-C).

Surface molecular composition: The molecular composition of *E. coli* cell surface was modeled in terms of polysaccharide (ps), proteins (pr) and hydrocarbon-like (HC). This molecular composition was computed with the following equations based on the three components of the carbon peak (Dufrene and Rouxhet, 1996b; Rouxhet *et al.*, 1994).

$$[(C = O/C)]_{obs} = 0.279\ (C_{pr}/C) + 0.167\ (C_{ps}/C)$$
$$[(C\text{-}(O, N))/C]_{obs} = 0.293\ (C_{pr}/C) + 0.833\ (C_{ps}/C)$$
$$[(C\text{-}(C, H)/C]_{obs} = 0.428\ (C_{pr}/C) + 1\ (C_{HC}/C)$$

Solving the systems of equations provides the proportion of carbon associated with each molecular constituent: (C_{pr}/C), (C_{ps}/C) and (C_{HC}/C). These proportions can be converted into weight fractions, using the carbon concentration of each model constituent.

RESULTS AND DISCUSSION

Surface physico-chemical properties of *E. coli*: Bacterial cell surface physic-chemical characteristics are presented in Table 1. In this study we have based on ΔGiwi to evaluate the degree of hydrophobicity of *E. coli* strains. From Table 1, the ΔGiwi varied from 7.52 mjm^{-2} (strains AL52 (LLB)) to 23.89 mjm^{-2} (strains 382 (SLB)). The positive value of ΔGiwi indicates that

Table 1: Contact angle of water (θ_w), formamide (θ_F) and diiodomethane (θ_D) and Lifshitz-van der Waals (γ^{LW}) and acid-base (γ^{AB}) components of the total surface and electron-donor (γ^-) and electron acceptor (γ^+) parameters and free energy interactions ($\Delta Giwi$)

	Contact angle (°)			Surface tension components and parameters (MJ m^{-2})				
								Free energy (MJ m^{-2})
E. coli strains	θ_w	θ_F	θ_D	γ^{LW}	γ^-	γ^+	γ^{AB}	$\Delta Giwi$
AL52 LLB	27.15 (3.1)	34.23 (5)	92.6 (4.83)	11.73	45.03	11.96	45.26	7.52
AL52 SLB	23.8 (2.73)	22.2 (3.01)	81.8 (3.05)	14.63	42.64	11.16	43.10	8.63
HB101 LLB	25.73 (3.75)	37.73 (2.57)	83.8 (2.36)	15.64	54.75	6.58	37.89	22.36
HB101SLB	20.15 (3.12)	25.35 (2.15)	75.85 (1.7)	19.85	52.35	7.10	38.10	20.77
382 LLB	36 (3)[a,b]	31 (0.02)[b]	72 (0.02)[b]	21.79	39.94	5.92	30.75	13.29
382 SLB	25 (2)[a,b]	27 (0.02)[b]	66 (0.02)[b]	25.18	49.89	4.25	29.14	20.07

Standard deviation was given in parentheses; a: From Latrache *et al.* (2002) and b: From Oliviero (1993)

Table 2: Elemental composition and functional composition of three *E. coli* cultivated under different conditions (atom fraction (%) excluding hydrogen; mean of at least two analyses of cells from independent cultures) (Latrache *et al.*, 1994)

	C-(C,H)	C-(O,N)	(C = O)	(O = C)	(-OH, C-O-C)					Non-prot-N	Prot-N
E. coli strains	284.8 eV	286.2 eV	287.9 eV	531.2 eV	532.6 eV	N/C	O/C	P/C	N/P	399.9 eV	401.6 eV
AL52 LLB	32.1	24.2	9.0	6.8	20.8	0.09	0.423	0.014	6.55	5.4	0.52
AL52 SLB	24.6	26.9	11.0	8.2	22.8	0.085	0.496	0.011	7.57	4.8	0.53
HB101LLB	36.5	20.1	8.6	10.6	16.2	0.101	0.418	0.023	4.33	5.8	0.72
HB101SLB	38.7	19.5	8.2	9.5	15.9	0.095	0.383	0.02	4.846	5.7	0.63
382 LLB	38.5	18.5	9.9	10.2	12.2	0.132	0.325	0.015	9.10	8.5	0.65
382 SLB	30.2	21.8	10.7	10.5	16.2	0.139	0.426	0.016	8.70	8.2	0.50

Table 3: Surface molecular composition of *E. coli* Hamadi *et al.* (2005)

E. coli strains	Hydrocarbon like (%)	Proteins (%)	Polysaccharides (%)
AL52 LLB	28.0	35.0	37.0
AL52 SLB	41.0	37.0	21.0
HB101 LLB	30.0	29.0	43.0
HB101 SLB	29.0	25.0	47.0
382 LLB	40.5	18.5	38.5
382 SLB	45.5	26.3	28.7

these different bacterial strains tested here were hydrophilic. The culture of cells on SLB or in LLB influences slightly the hydrophilicity of Al52 and HB101 strains and influences strongly the hydrophilicity of 382 strains. It has been reported that the degree of hydrophobicity was related to the surface chemical composition, surface molecular composition and their external structures (El-Ghmari *et al.*, 2002; Fatima *et al.*, 2005; Latrache *et al.*, 2002). This may explain the difference observed on the degree of hydrophobicity ($\Delta Giwi$) between *E. coli* strains and between cells cultivated in different medium. From Table 1 it can also be observed that all strains have surfaces with predominately electron donor (high values of γ-) with a low electron acceptor (γ+). These properties varied with the culture medium used. Indeed, we found that the electron donor property of AL52 and HB101 strains cultivated in SLB medium was less important compared to cells cultivated in LLB medium. In contrast, the electron donor property of strain 382 cultivated in LLB medium was more important compared to cells cultivated in SLB medium. AL52 and 382 strains cultivated in LLB medium were found to be the least electron accepting compared to the cells cultivated in SLB. The difference observed in these results would be discussed in term of surface chemical composition and surface molecular composition (Table 2, 3).

Relation between surface chemical composition and electron donor/electron acceptor properties (Acid-base properties): The electron donor/electron acceptor (acid-base) properties play an important role in various interfacial phenomenons such as adhesion (Henriques *et al.*, 2004; Hamadi *et al.*, 2005; Hamadi *et al.*, 2008; Van Oss, 1993). Three methods including Microbial adhesion to solvent (Bellon-fontaine *et al.*, 1996), contact angle measurements combined with the equation of Van Oss *et al.* (1988b) and acid-base titration (Borrok *et al.*, 2004; Cox *et al.*, 1999; Fein *et al.*, 1997; Hong and Brown, 2006) have been used to determine the acid- base properties of cell surfaces. The works based on acid-base titration method have calculated the acidity constants (pKa) using different models such as FITEQL (Hong and Brown, 2006) and surface complexation model (Fein *et al.*, 1997). From the pka values they have reported that, generally, the acid-base properties originates from functional groups presents on the cell surface including carboxylic, phosphoric hydroxyl and amine groups. They also determined the number and the density of acid-bases groups. The determination of these acid-base groups and their number have used mainly to explain the origin of cell surface charge (Hong and Brown, 2006) and to understand the metal-bacterial interactions (Fein *et al.*, 1997; Yee and Fein, 2001) but they doesn't used to explain the origin of acid-base properties. In our knowledge, limited data (Sharma and Hanumantha, 2002) have attempted to relate the acid-base properties evaluated by contact angle measurements and the elemental chemical composition determined by XPS. These works have found that there is no direct correlation between the electron donating of gram negative bacterial cell and elemental chemical composition. In this study we have based on surface chemical composition and surface molecular composition given by XPS and modeling XPS data respectively to determine the origin of electron donor/electron acceptor (acid-base) properties evaluated by contact angle measurements combined with the equation of Van Oss *et al.* (1988b). The correlation between surface chemical properties and electron donor electron acceptor (acid-base) properties were examined. Figure 1 shows the correlation ($r = 0.84$) between electron donor (base property) and P/C ratio.

These results indicate that phosphate groups in basic form play a predominant role in determining the electron donor property (base) of *E. coli*. These results are explained with the fact that phosphate groups are constituents of phospholipids and lipopolysaccharides in gram negative bacteria (Beveridge, 1981; Beveridge, 1988). Our finding are not in agreement with works of, Sharma and Hanumantha (2002) which reported that Gram-negative bacteria cells having higher electron-donor parameter had lower nitrogen, oxygen and phosphorous content on their cell surfaces.

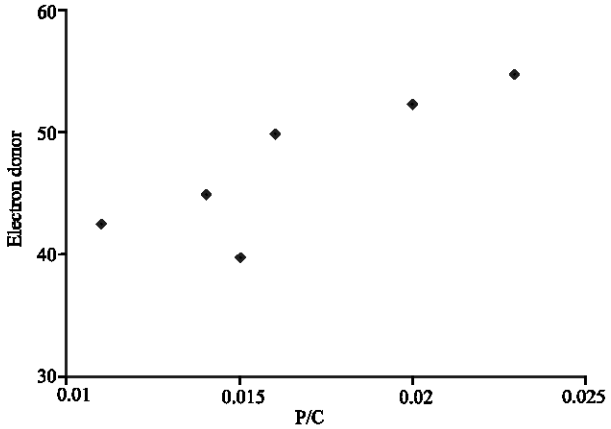

Fig. 1: Relation between electron donor property and P/C ratio

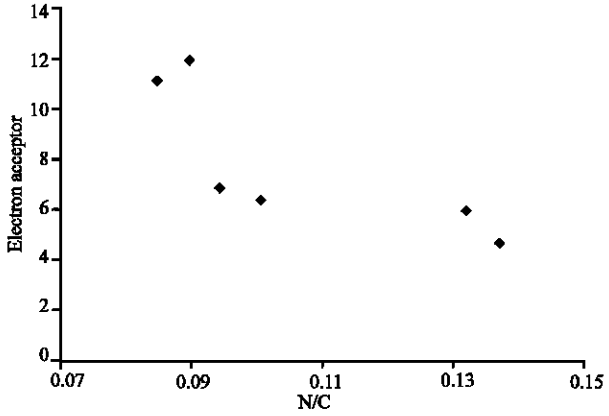

Fig. 2: Relation between electron acceptor property and N/C ratio

A negative correlation (r = -0.82) between electron acceptor (acid) and N/C ratio was found (Fig. 2). This indicates that the nitrogen plays an important role in decreasing the electron acceptor (acid) property. It should be pointed out that this correlation involves the total surface concentration of nitrogen and not only a concentration of protonated nitrogen or non-protonated nitrogen. According to Table 2, the protonated nitrogen contributes by a small fraction of total nitrogen of the cell surface. Therefore, we can suggest that low concentrations of nitrogen in the form non protonated are responsible of high electron acceptor (acid) property.

The decomposition of XPS peaks provides information about the functional composition of the cell surface. We have also interested to relate these functional compositions to acid-base properties in order to complete the results obtained previously (Hamadi *et al.*, 2008) reporting that these functional compositions play a role in determining the hydrophobicity and surface charge of *E. coli*.

No clear relation was observed between functional composition and electron donor property. The relation between electron acceptor (acid) property appear to be directly correlated with (OH, C-O-C) function and inversely correlated with (O = C) function (Fig. 3a-b). Since (OH, C-O-C) function and (O = C) function were considered as indicators of the presence of polysaccharides and proteins respectively (Cuperus *et al.*, 1993; Hamadi *et al.*, 2008) we can hypothesis that higher electron acceptor property could be accompanied by a loss amount of proteins and large amount of polysaccharides. These results are confirmed when we have examined the relation between the acid-base properties and the molecular composition. This molecular composition have determined by converting the surface chemical composition given by XPS in terms of proteins, polysaccharides and hydrocarbonlike. According to Fig. 4, we observe that as the polysacchrides concentration increased the surface became more electron acceptor (Fig. 4a). This indicates that polysaccharide contributes to electron acceptor property of *E. coli* strains. This is completely in agreement to our hypothesis concerning the role of the functional composition in acid-base properties.

Moreover, the ratio of polysaccharides/proteins is seen to correlate positively with the electron acceptor property (Fig. 4b). This observation suggests that the electron acceptor property increase with increasing of polysaccharides and with decreasing of proteins. In this work, we haven't show a direct correlation between electron acceptor property and proteins concentration (Table 3) but we have obtained that this property decrease with N/C ratio (Fig. 2) and with (O = C) function (Fig. 3a). These parameters were indicators of the presence of proteins. So we could suggest that low electron acceptor property was associated with the presence of proteins.

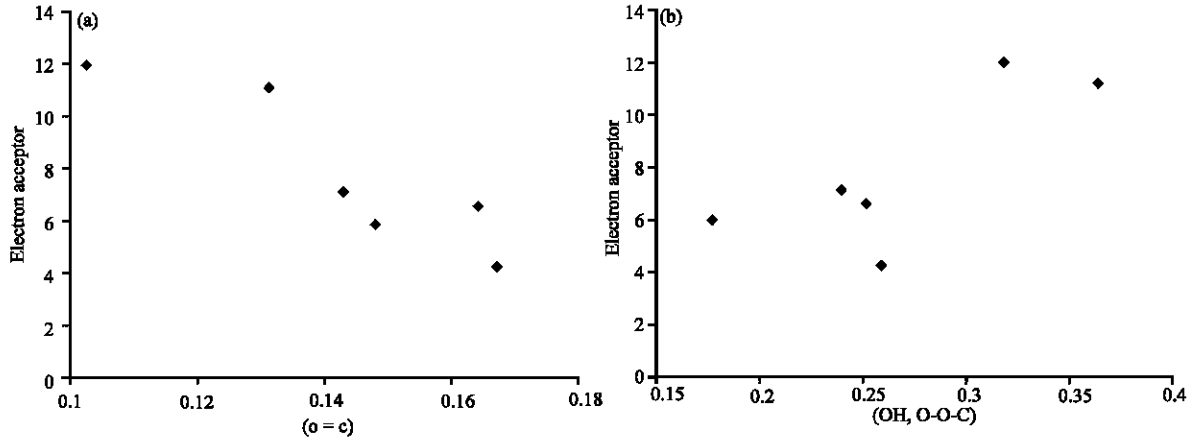

Fig. 3(a-b): Variation of electron acceptor property as a function of surface functional composition: (a) (O = C) and (b) (OH, C-O-C)

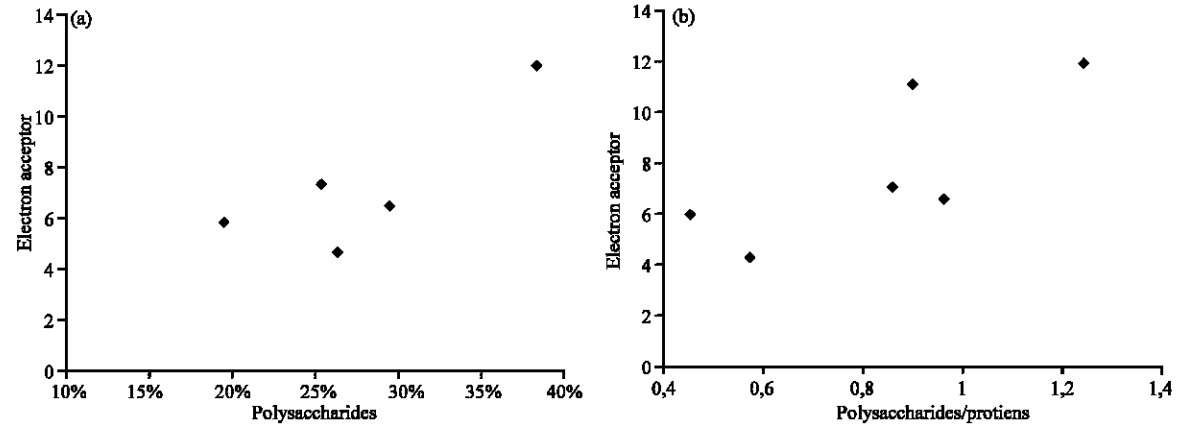

Fig. 4(a-b): Variation of electron acceptor property, (a): As a function of polysaccharides and (b): Polysaccharides/proteins ratio

CONCLUSION

This study presents an attempt to relate the electron donor electron acceptor (acid-base) properties to their surface chemical composition and their surface molecular composition. The finding in this work reported that phosphate groups clearly contribute to enhanced electron donor property and electron acceptor property is linked to a low concentration of amine groups. Also the electron acceptor property is in agreement with high concentration of polysaccharide and low concentration of proteins.

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