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Effect of pH and ionic strength on hydrophobicity and electron donor and acceptor characteristics of *Escherichia coli* and *Staphylococcus aureus*

F. HAMADI[1], H. LATRACHE[1*], A. EL GHMARI[1], M. ELLOUALI[1], M. MABRROUKI[2], N. KOUIDER[1]

[1]Laboratoire de Valorisation et Sécurité des Produits Alimentaires;
[2]Laboratoire de Physique des Matériaux, Faculté de Sciences et Techniques, Beni Mellal, B.P 523, Morocco

Abstract - The aim of this study was to use M.A.T.S (Microbial adhesion to solvents) in order to examine the influence of pH and ionic strength on electron donor/electron acceptor properties and surface hydrophobicity of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* AL52. Using this technique we found that the electron donor character for both strains was influenced by pH and ionic strength. This effect was more important for *S. aureus*. The electron acceptor was expressed for acidic pH. Regardless of pH, the electron donor character of *S. aureus* changed when the ionic strength increased from 10^{-3} M to 10^{-1} M. For *E. coli*, the electron donor character varied with ionic strength at pH 2, nevertheless, for other pH, this character was not influenced by ionic strength. Moreover, surface hydrophobicity of two microbial cells surface was affected by pH and ionic strength. It was maximal at acidic pH and lower at basic pH for *S. aureus*. Regardless of pH and ionic strength, *E. coli* was hydrophilic**.**

Key words: microbial cell surface, hydrophobicity, electron donor/electron acceptor properties, pH, ionic strength, M.A.T.S.

INTRODUCTION

Microbial adhesion to solid surfaces depends on reversible and subsequently irreversible interactions (Beachey, 1981; Busscher and Weerkamp, 1987; Mozes *et al*., 1991; Ofek and Doyle, 1994). The reversible initial stage results from complex physicochemical interactions among the cell, the surface and the liquid phase (Kim and Frank, 1994). These interactions are caused by the surface charge (Hogt *et al*., 1985; Dickson and Koohamaraie, 1989), the hydrophobicity (Dahlback *et al*., 1981; Van Loosdrecht *et al*., 1987) and electron acceptor and electron donor (Van Oss, 1993) of interacting surfaces.

The role of electron-donor/electron acceptor, i.e. Lewis acid-base proper-

*Corresponding Author. E-mail: latracheh@yahoo.fr

ties, in the interaction between two materials has been widely studied (Van Oss and Visser, 1992). Their importance in polar aqueous media has been underlined and reviewed by Van Oss (1993). Several studies (Boulangé-Petermann *et al*., 1993; Van Oss, 1993) have reported that the electron-donor/electron acceptor plays a crucial role in the microbial adhesion phenomenon. It should be noted that the energy of these interaction may be twice as much as that produced by the Lifshitz-van der Waals interactions (LW) or electrostatic interactions (EL) usually described in the DLVO theory (Van Oss, 1996).

In 1996, Bellon-Fontaine *et al.* developed a new method-namely M.A.T.S (Microbial adhesion to solvents), to determine the electron donor/electron acceptor microbial cell properties. It was based upon the comparison between microbial cell affinity to a monopolar solvent and a polar solvent with the same LW surface tension component. This technique appears to be more useful than contact angle method (Van Oss *et al*., 1988), which require specific and elaborate equipment.

Microbial cell surface hydrophobicity is recognized as one of the determinant factors in microbial adhesion to surface (Van Loosdrecht *et al*., 1987). These properties are often evaluated by hydrophobic interaction chromatography, contact angle method, aqueous phase partitioning poly-ethyleneglycol/dextran (PEG/DEX) and microbial adhesion to hydrocarbon (M.A.T.H). The latter technique is generally performed using p-xylene, hexadecane, octane and toluene. So, it can be a useful method to measure the cell surface hydrophobicity.

The cell surface physicochemical properties can be modified depending on surface cell structures (Ljunjh and Wadstrom, 1984; El Ghmari *et al*., 2002) or environmental factors such as temperature, medium composition, ionic strength and pH. Many workers have described the effects of these environmental parameters on hydrophobicity and charge (Beck *et al*., 1988; Herben *et al*., 1990; Van Der Mei *et al*., 1993; Latrache *et al*., 1994; Braindet *et al*., 1999a; Latrache *et al*., 2000). Literature data (Rouxhet and Mozes, 1990) reported that the hydrophobicity and charge were insufficient to explain the adhesion phenomenon. So the involvement of electron donor/electron acceptor properties could also be important in explaining this phenomenon (Van Oss *et al*., 1988).

Despite the fact that the electron donor/electron acceptor properties play an important role in adhesion phenomenon, limited data concerning the effects of environmental parameters on these properties have been published (Braindet *et al*., 1999a; 1999b). To our knowledge, no study has been performed on electron donor/electron acceptor properties under different pH and ionic strength.

The aim of this investigation was to use M.A.T.S, a simple, rapid and quantitative method, in order to study the influence of pH and ionic strength mainly on electron donor/electron acceptor properties (i.e., the Lewis acid properties) of microbial cell surface and secondly on the surface hydrophobicity. This study has been carried out on two bacteria, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* AL52, chosen as the test microorganism for their capacity to adhere to inert surface, such as catheters (Gross *et al*., 2001; Dankert *et al*., 1986), causing several infections.

MATERIAL AND METHODS

Bacterial strains, growth conditions and preparation of microbial suspension. Two bacteria were studied: *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* AL52, isolated from patient with urinary tract infections.

For each growth conditions, bacteria were subcultured overnight and grown in solid Luria–Bertani at 37 °C for 24 h. After culture, the cells were harvested by centrifugation for 15 min at 8400 *x g*, washed twice and resuspended in the suspending liquid (KNO_3, 10^{-3} or 10^{-1} M). KNO_3 and chemical products (chloroform, hexadecane, diethyl ether and hexane) having a highest purity grade were obtained commercially.

M.A.T.S: **Microbial Adhesion To Solvents**. The partitioning method previously described by Bellon-Fontaine *et al*. (1996) is based on the comparison between microbial cell affinity to a monopolar solvent and an apolar solvent. The monopolar solvent can be acidic (electron accepting) or basic (electron donating) but both solvents must have similar Lifshitz-van der Waals surface tension components. On this basis, the following pairs of solvents were selected: a) chloroform, an acidic solvent (electron acceptor) with hexadecane, an apolar solvent; b) diethyl ether, a strongly basic solvent (electron donor) and hexane, an apolar solvent.

Experimentally, the bacteria were suspended to an optical density at 405 nm (A_0) of 0.7- 0.8 in KNO_3 10^{-3} or 10^{-1} M, with the pH adjusted to 2, 3, 5, 9, 11 by the addition of HNO_3 or KOH. Next, 0.4 ml of the solvent was added to 2.4 ml of bacterial suspension, after which the two phase system was vortexed for 90 s and allowed for 15 min to ensure complete separation of the two-phases (organic and water phase). The optical density (A) of water phase was measured.

The percentage of bound cells was subsequently calculated by the formula:

$$\% \text{ Adherence} = (1 - A/A_0) \times 100$$

where A_0 is the optical density measured at 405 nm of the bacterial suspension before mixing.

Each experiment was performed in triplicate by using three independently prepared cultures.

RESULTS AND DISCUSSION

Influence of pH and ionic strength on the electron donor character of microbial cell surface

The electron donor/acceptor properties were estimated by the comparison of cell affinity to monopolar and apolar solvents that have similar Lifshitsz-van der Waals surface tension components. The electron donor character was estimated by the difference between affinity to chloroform and hexadecane (Bellon-Fontaine *et al*., 1996).

The M.A.T.S results obtained for *S. aureus* strain and *E. coli* strain under different pH and ionic strength are shown in Table 1. Regardless of pH and ionic strength, the affinity of *S. aureus* strain was always higher with chloroform (an electron acceptor solvent) than with hexadecane (apolar solvent). These results

TABLE 1 – Affinities of *Staphylococcus aureus* and *Escherichia coli* cells for the two solvents used in the M.A.T.S analysis under different pH and ionic strength

Ionic strength	pH	% Affinity with solvents			
		S. aureus		*E. coli*	
		Chloroform	Hexadecane	Chloroform	Hexadecane
10^{-3} M	2	94 (7)	95 (3)	74.5 (6)	26 (1)
	3	98 (1)	76 (18)	0 (0)	3 (3)
	5	85 (11)	22 (2)	0 (0)	1 (1)
	6.2 (6.9)*	27 (5)	4 (2)	0 (0)	0 (0)
	9	17 (16)	1 (1)	0 (0)	0 (0)
	11	95 (2)	17 (6)	0 (0)	0 (0)
10^{-1} M	2	100 (0)	72 (14)	42.5 (0)	13 (1)
	3	99 (1)	63 (32)	1 (1)	7 (0)
	5	99 (1)	42 (9)	6 (4)	4 (4)
	6.2 (6.9)*	83 (20)	29 (9)	2 (2)	6 (4)
	9	94 (4)	39 (8)	4 (2)	5 (4)
	11	89 (10)	75 (13)	7 (7)	3 (3)

The standard deviation is given in parentheses. *pH value refereed to Escherichia coli.

indicate the electron donor character of *S. aureus* strain. For pH 2, *E. coli* strain exhibited higher affinity with chloroform than with hexadecane. These results showed that *E. coli* strain was electron donating only for this pH.

Unlike *E. coli*, *S. aureus* was generally an electron donor. The electron donor character here can be attributed to the presence of basic groups in the cell surface (Bellon-Fontaine *et al*., 1996; Braindet *et al*., 1999b), such as carboxyl (COO^-), phosphate (PO_4^{2-}) and amino groups (NH_2). The origin of difference between *S. aureus* and *E. coli* comes from the elemental composition. At neutral pH staphylococci have a higher N/C (0.15-0.20) due to the presence of amine groups and O/C (0.45-0.55) showing the presence of carboxyl groups (Van der Mei *et al*., 1989). While *E. coli* presents lower N/C (0.03-0.09) and O/C (0.28-0.43) (Harkes *et al*., 1992).

As shown in Fig. 1, at high ionic strength, *S. aureus* strain presents a clear maximum of electron donor character from pH 5 to pH 9. In both limits acid pH (pH 2, pH 3) and basic pH (pH 11) the electron donor character was relatively low (Fig. 1A). At low ionic strength, maximum electron donor character of *S. aureus* strain was seen for pH 5 and pH 11, but low for other pH values (Fig. 1A).

At high or low ionic strength, *E. coli* strain showed the maximum electron donor character at most acidic pH (pH 2) but for other pH this character was null or negligible (Fig. 1B).

Braindet *et al.* (1999a) have reported that a slightly acidic condition decreased the electron donor properties of *Listeria monocytogenes*. In this work

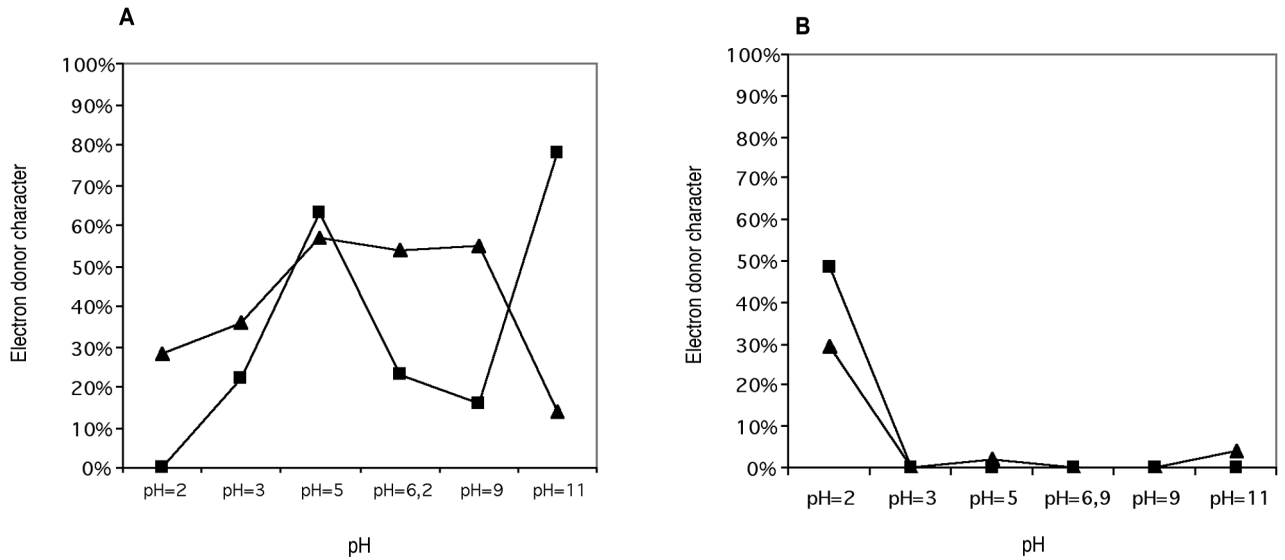


FIG. 1 – Electron donor character (difference between microbial adhesion to chloroform and microbial adhesion to hexadecane) of *Staphylococcus aureus* (A) and *Escherichia coli (B)* as a function of pH and ionic strength. —▲— 0.1 M, —■— 0.001 M.

we found that the reduction of electron donor appeared at most acidic pH (pH 3 to pH 2) while at slightly acidic pH (pH 6 to 5) this character increases. The random behaviour could be related to the presence of functional groups that could be strongly donating or weakly accepting according to pH.

The influence of the ionic strength of the suspending liquid was tested. Expect for pH 5 and pH 11, the electron donor character of *S. aureus* increased with the ionic strength increased (Fig. 1A). The inhibition of electrostatic interactions at high ionic strength could explain this phenomenon. Also, Harkes *et al.* (1992) obtained a good correlation (r = 0.84) at neutral pH between acid-base component of the surface free energy and zeta potential, indicating that acid/base interactions will play an important role when there is less negative charge on the bacterial surface. This could explain the effect of ionic strength observed here. Conversely, for *E. coli* this character increased when the ionic strength decreased at pH 2, but for other pH it appeared that the ionic strength did not affect this character (Fig. 1B). We have no rigours explanation for this type of behaviour for *E. coli* at pH 2 and for *S. aureus* at pH 5 and pH 11.

In order to study deeply the behaviour of microbial cell surface under different pH, it is necessary to investigate the properties of polar solvent (like chloroform) under different pH.

Influence of pH and ionic strength on electron acceptor character of microbial cell surface

Table 2 shows the affinities with diethyl ether and hexane used in M.A.T.S method of two microbial cell surfaces under different pH and ionic strength. The

TABLE 2 – Affinities of *Staphylococcus aureus* and *Escherichia coli* cells for the two solvents used in the M.A.T.S analysis under different pH and ionic strength

Ionic strength	pH	% Affinity with solvents			
		S. aureus		*E. coli*	
		Diethyl ether	Hexane	Diethyl ether	Hexane
10^{-3} M	2	98 (3)	89 (10)	0 (0)	34 (2)
	3	48 (9)	44 (13)	4 (4)	11 (1)
	5	3 (2)	70 (2)	2 (2)	1 (1)
	6.2 (6.9)*	3 (1)	14 (2)	0 (0)	0 (0)
	9	0 (0)	0 (0)	0 (0)	0 (0)
	11	7 (2)	25 (4)	1 (1)	0 (0)
10^{-1} M	2	53 (19)	56 (24)	0 (0)	19 (1)
	3	47 (14)	60 (32)	6 (4)	6 (0)
	5	5 (4)	54 (8)	9 (1)	3 (3)
	6.2 (6.9)*	10 (10)	34 (7)	8 (2)	4 (0)
	9	8 (4)	53 (10)	8 (2)	3 (1)
	11	11 (5)	27 (7)	14 (2)	4 (2)

The standard deviation is given in parentheses. *pH value referred to Escherichia coli.

electron acceptor character was estimated by the difference between affinity to diethyl ether and hexane but only positive values were considered (Bellon-Fontaine *et al*., 1996).

At low ionic strength and only acidic pH (pH 2, 3), bacterial affinity of *S. aureus* strain was higher with diethyl ether (a strongly electron-donating solvent) than with hexane (apolar solvent). These results show the electron acceptor properties of *S. aureus* strain for same acidic pH. Likewise, *E. coli* exhibited higher affinity with diethyl ether than with hexane for acidic pH (pH 2, 3) and at high ionic strength indicating that *E. coli* strain was an electron acceptor for these pH and this ionic strength.

According to these results, it appears that both strains were an electron acceptor only for pH 2 and pH 3. At others pH values, both microbial cell surfaces showed a very weak electron acceptor character.

Generally, numerous previous studies (Bellon-Fontaine *et al*., 1996; Pelletier *et al*., 1997; Braindet *et al*., 1999a, 1999b) on the estimation of electron donor/electron acceptor properties of microbial cell surface at neutral pH have shown that these microbial cell surfaces were strongly donating and weakly accepting. It should be noted that the electron acceptor character could be more expressed at other pH especially in acidic pH. At acidic pH the functional groups were deprotonated, this could explain the electron acceptor character obtained for acidic pH.

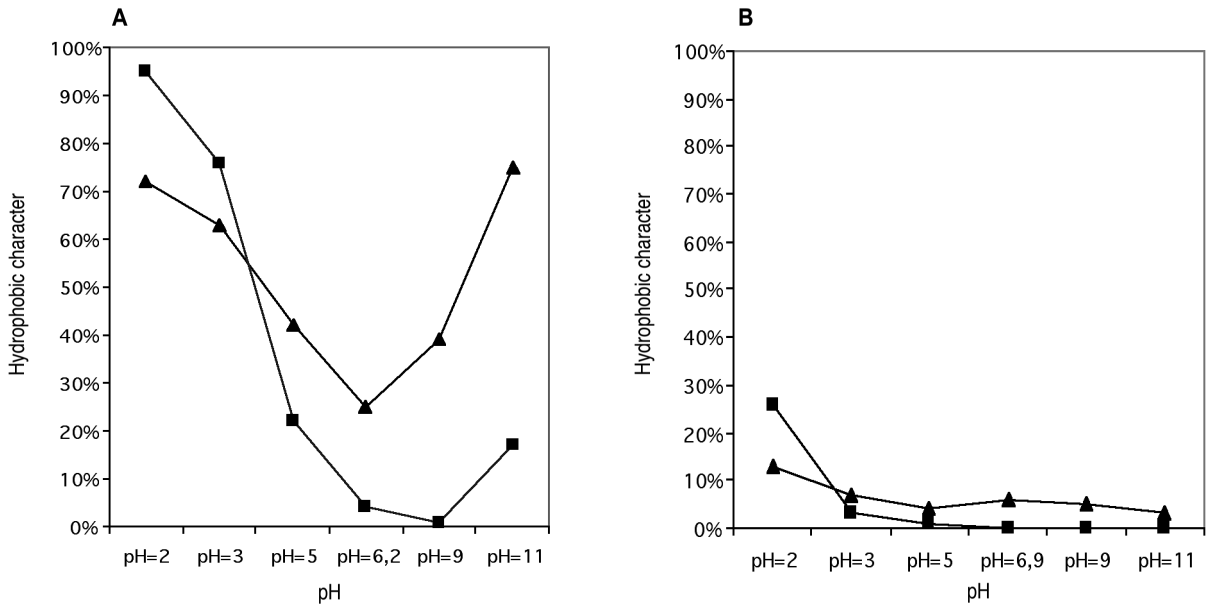
Few studies have determined the electron donor/electron acceptor properties of microbial cell surface using M.A.T.S. These studies have reported that these properties could be attributed to functional groups on the surface. So to confirm this hypothesis and to investigate the involvement of others components in theses properties, further work must be realised on the relation between the surface chemical composition determined by XPS, the biochemical composition and the electron donor/electron acceptor properties.

Influence of pH and ionic strength on hydrophobic character of microbial cell surface

The hydrophobicity of microbial cell surfaces was estimated by measuring the percentage of cell adhering to hexadecane.

Figure 2 presents the evolution of hydrophobic character as a function of pH and at two ionic strengths of microbial cell surfaces. *Staphylococcus aureus* displayed a maximal hydrophobic character at pH 2 (higher affinity to hexadecane, Table 1). This character decrease when pH increases for both ionic strength expect in pH 11 where this character was still important at 10^{-1} M (Fig. 2A).

However, it has been shown that M.A.T.H measures a complicated interplay of hydrophobic and electrostatic interactions (Van der Mei *et al*., 1993). The hydrocarbons in suspension are hydrophobic and negatively charged (Medrzycka, 1991; Geertsema-Doornbusch *et al*., 1993; Busscher *et al*., 1995). The repulsive electrostatic interactions could prevent the adhesion to hexadecane (Busscher *et al*., 1995; Van der Mei *et al*., 1995). Consequently only strains with low negative surface charge expressed the maximal hydrophobic interactions to hexadecane (Busscher *et al*., 2000). It has been known that the negative charge measured by electrophoretic mobility becomes more negative when increasing pH. Thus, in acidic pH, the repulsive interactions are very weak making hydro-



F IG. 2 – Hydrophobic character (percentage of adhesion to hexadecane) of *Staphylococcus aureus (A)* and *Escherichia coli (B)* as a function of pH and ionic strength. —▲— 0.1 M, —■— 0.001 M.

phobic interactions becomes more important. This explains, in the case of 10^{-3} M, the maximum hydrophobicity observed at pH 2 and pH 3, and the relative hydrophilic character observed from pH 5 to pH 11 for *S. aureus* strain (Fig. 2A).

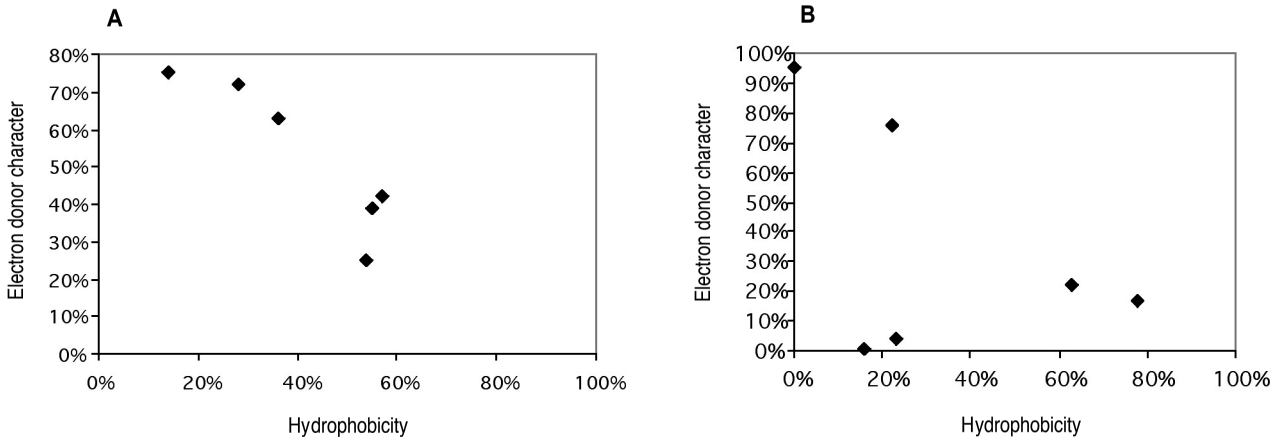
These results are in accordance with data published by Van der Mei *et al.* (1993), which indicated that thermophilic dairy streptococci exhibited a maximum hydrophobicity for the pH range from pH 2 to pH 4. Mafu *et al*. (1991) found that the hydrophobicity of *L. monocytogenes S*cott*,* measured by hydrophobic interaction chromatography technique, increased when the pH decreased. The classical DLVO theory predicts that microorganism below their isoelectric point, i.e. bearing a positive surface charge, should adhere better to hydrocarbons than suspended aqueous solutions above their isoelectric point (Bos *et al*., 1999).

The influence of ionic strength on hydrophobicity was examined. Expect for pH 11 at 10^{-1} M, the hydrophobicity as a function of pH shows the same profile for both ionic strengths (Fig. 2A). Moreover, the variation of ionic strength from 10^{-3} M to 10^{-1} M showed a reduction of cell surface hydrophobicity at acidic pH (pH 2, 3) but inversely, from pH 5 to pH 11, this hydrophobicity was higher in 10^{-1} M (Fig. 2A).

Generally, since the bacterial cell surface were positive at very acidic pH and negative at other pH, the increase of hydrophobicity could explain by the reduction of repulsive negative electrostatic interactions at 10^{-1} M and the reduction of hydrophobicity could be explained by the reduction of repulsive positive electrostatic interaction at 10^{-1} M.

For *E. coli*, regardless of the pH and ionic strength, the microbial cell surface was hydrophilic (lower affinity to hexadecane, Table 2). This character becomes more hydrophilic when increasing pH (Fig. 2B). Expect for pH 2, the hydrophilic character was not influenced by the ionic strength (Fig. 2b). In the case of hydrophilic strains, the Van der Waals attraction between cells and water is so much stronger than any electrostatic interactions between cells and hexadecane (Van der Mei *et al*., 1993). This explains the independence of hydrophilic character of *E. coli* strain as a function of pH (Fig. 2B). The hydrophilic character of *E. coli* was found previously with using the hydrophobic interaction chromatography (HIC) (Latrache, 1993); the contact angle and adhesion to paraxylene were used (Latrache *et al*., 2002).

Finally, the correlation between electron donor properties and hydrophobicity, percentage of adhesion to hexadecane, was examined (Fig. 3). For *S. aureus* strain, a good correlation (r = 0.91) was obtained between those properties at 10^{-1} M indicating that the electron donor character decreases when the hydrophobicity increases, the same result was obtained by Wu *et al.* (1994) which observed that smectite and other mineral particles became more hydrophobic owing to a decrease in the electron donating surface tension parameter. Conversely, there is no correlation (r = 0.45) between hydrophobicity and electron donor character at low ionic strength, we think that the absence of correlation could be due to the two points (pH 9 and 11). In comparison with high ionic strength, the electrostatic interactions were strongly important at pH 9 and pH 11 for low ionic strength. It seems to be that the correlation between hydrophobicity and electron donor character could be obtained only with weak electrostatic interactions. For *E. coli* strain there is no correlation between hydrophilicity and electron donor character for both ionic strengths.



F IG. 3 – Relation between hydrophobicity and electron donor character of *Staphylococcus aureus*. (A): ionic strength 10^{-1} M, r = 0.91; (B): ionic strength 10^{-3} M, r = 0.45.

In conclusion the results presented here demonstrate the influence of pH and ionic strength on physicochemical properties (hydrophobicity; electron donor/electron acceptor) of *Staphylococcus aureus* and *Escherichia coli*. These results could contribute to understand microbial adhesion of both microbial cells to inert surface. The assay adhesion of *S. aureus* to inert surface at different pH carried out in the laboratory show a good correlation between the number of adhering bacteria and the physicochemical properties determined here, results coming in new paper using scanning electron microscopic (S.E.M.) images and program in mathlab processing image modules (data unpublished).

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