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Adhesion of *Escherichia coli* to Glass Under Different pH

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The hydrophobic/hydrophilic and electron donor/electron acceptor characters for *Escherichia coli* AL52 were determined using microbial adhesion to solvents method (MATS). The glass with adhered cells was observed using scanning electron microscope (SEM) and the SEM images were processed with Mathlab® program to quantify the adhesion to glass. Strong adhesion was found at pH 2. For pH 3 the adhesion was relatively high but for other pH it was low or null. The results also show that the electron donor character plays a crucial role in microbial adhesion of *Escherichia coli* to glass. Moreover the role of hydrophilic-hydrophilic interactions is not always sufficient to explain adhesion between two hydrophilic surfaces. Also our finding shows that the types of interactions governing microbial adhesion to glass depend on pH of suspending medium.

Key words: *Escherichia coli*, Adhesion, pH, electron donor character, hydrophobicity.

Bacterial adhesion to surface is ubiquitous and known to form the basis for several diverse problems in medicine[1-4], industry[5-8] and environmental areas[9,10].

Escherichia coli are a major cause of catheter associated urinary tract infection (UTI) and the most common noscomial pathogen[1,11]. The adhesion of *Escherichia coli* to catheter surface was considered as the first step of biofilm formation and consequently urinary tract infection. By studying the adhesion mechanisms we gain insight into processes such as the initiation of infection[12,13] and biofilm formation[14, 15]. A better understanding of bacterial adhesion requires good knowledge of the interactions involved in this phenomenon. These interactions depend on physico-chemical properties of the substratum, the suspending medium and the bacterial surfaces such as hydrophobicity, surface charge and electron donor/electron acceptor characteristics[16-20].

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The interaction between bacteria and solid surface is often described by the Derjaguin-Landan-Verwey-Overbeek (DLVO) theory for colloidal stability. In this model only electrostatic and Van der Waals forces explained the interaction between bacteria and solid surface[21,22]. However, the DLVO model is often not sufficient to explain bacterial adhesion[23,24]. So, Van Oss, suggested to include the acid- base interactions in the DLVO theory[25]. According to Van Oss, the acid –base interactions are 10~100 times important than the other interactions.

Despite the importance of Lewis acid base interactions in the explanation of bacterial adhesion, most previous work investigated the role of hydrophobicity and surface charge in the adhesion of cells[17,26-35] but few studies have focused on the role of electron donor/electron acceptor character (Lewis acid-base properties) in bacterial adhesion[18, 36 - 38]. In this work we discuss two aims. The first aim was to investigate the adhesion of *E. coli* AL52 strain to glass under different pH. The glass was chosen in this study as a model substratum. The second aim was to evaluate the role of cell surface properties especially the electron donor property in bacterial adhesion.

MATERIAL AND METHODS

Bacterial strain and culture conditions.

The *Escherichia coli* strain Al52 which isolated from patients with urinary tract infections was used in this study. Bacteria were grown overnight at 37°C in Luria–Bertani agar containing the following (per litre of distilled water): 10 g of tryptone, 5 g of yeast extract, 10 g of NaCl and 15 g of agar. After culture, cells are scraped form solid medium and are suspended in a solution of KNO_3 and were harvested by centrifugation for 15 min at 8.400 × g and washed twice with and resuspended in 0.1 M KNO_3 solution.

Microbial Adhesion to Solvents (MATS)

Hydrophobicity and electron donor/ electron acceptor (Lewis acid–base properties) of *E. coli* strain were assessed by aqueous partitioning assays, using the microbial adhesion to solvents (MATS)[39]. Briefly, bacteria were suspended to an optical density of 0.7 and 0.8 at 405 nm (OD_0) (approximately 10^8 CFU ml^{-1} cell density) in 0.1 M KNO_3, at various pH values 2, 3, 5, 9 by the addition of HNO_3 or KOH). The high concentration in electrolyte was used to avoid charge interferences, since it has been reported that some solvent droplets, especially hexadecane, are negatively charged in aqueous suspensions[40]. 2.4 ml volume of cell suspension was added to 0.4 ml of solvent. After, the two phases were vortexed for 90 s and allowed for 15 min to ensure complete separation of the two phases (organic and water phases). The optical density (OD) of water phase was measured. The percentage of microbial adhesion to solvent was calculated as $(1- OD/OD_0) \times 100$.

Four solvents were tested in this study: Hexadecane (apolar solvent), Chloroform (acidic solvent), Diethyl ether (Basic solvent) and hexane (apolar solvent).

Microbial adhesion to hexadecane reflects cell surface hydrophobicity or hydrophilicity because electrostatic interactions are absent as noted above. The differences between the results obtained with chloroform and hexadecane, on the one hand, and between diethyl ether and hexane, on the other hand indicate the electron–donor and electron–acceptor character of the bacterial surface, respectively.

Characteristics of solid surface.

The Lifshitz-Van der Waals (g^{LW}) component and the electron donor (g^-), and electron acceptor (g^+) surface energy parameters of glass were determined by measuring contact angle by using the approach proposed by Van Oss *et al.*[41]. In this approach, where spreading pressure is ignored, the contact angleq, measured with a pure liquid (L), is expressed as:

$\cos\theta = -1+2(\gamma_s^{LW} \gamma_L^{LW})^{1/2}/\gamma_L +2(\gamma_s^+ \gamma_L^-)^{1/2}/\gamma_L +2(\gamma_s^- \gamma_L^+)^{1/2}/\gamma_L$.

Three liquids (one nonpolar and two polar) are used. Thus three equations are obtained which are solved to obtain g^{LW} component and γ_s^- and $g\gamma_s^+$ parameters for the solid substrate.

The three pure liquids used were water (Milli-Q plus), formamide and diodomethane.

Substrate preparation

The solid support selected for this study was glass. The glass samples were microscope slides (Knittel Glazer, Germany). Before beginning adhesion assays, the glass samples were cut into square chips (1x1 cm) and cleaned by

soaking for 15 min in ethanol and rinsing six times with distilled water. Finally, the solid support was autoclaved for 15 min at 120.

Adhesion experiments

The cells were suspended in a 0.1 M KNO_3 solution to give an absorbance between 0.7 and 0.8 (approximately 10^8 CFU ml^{-1} cell density). The pH range of a suspension was 2 to 9. For each pH, 10 ml of bacterial suspension was incubated in a covered flask containing the glass substrate for 3 h at 25. The non-adhering bacteria were removed by rinsing the substrate four times with sterile water.

Scanning electron microscopy and Image analysis

The glass with adhered cells was dried with free air, metalized and observed using scanning electron microscopy (SEM). All SEM images were processed with Mathlabâ program to determine the percentage of glass surface covered by the cells. We use a development algorithm identifying the boundaries in image, based on some mathematical methods, exploring also image to detect edges and using statistical functions to calculate mean and standard deviation.

RESULTS AND DISCUSSION

Physico-chemical characterization of cells and solid surfaces

Bacterial surface hydrophobicity.

The cell surface hydrophobicity was determined from microbial adhesion to hexadecane. The results of cell surface hydrophobicity of *E. coli* AL52 were presented in Table 1. Whatever pH of microbial suspension, the microbial cell surface of *E. coli* strain exhibited very low affinity to hexadecane (apolar solvent). These results demonstrated the most hydrophilic property of this strain. The hydrophilic property of *E. coli* has previously been obtained by other reports using different methods[42- 46].

Electron donor/electron acceptor properties of *E. coli*

It has been known that the electron donor/ electron accepror properties were attributed to the presence of carboxyl and phosphate groups at the cell surface[37, 39]. Since these groups depend on pH of suspending medium, the electron donor/ electron acceptor may be changed with pH. So it is interesting to examine these properties with pH. The Results of the electron donor/electron acceptor properties of cell surface of *E. coli* are presented in Table 1. At pH 2, the affinity of cell surface was high with chloroform (acidic solvent) than with hexadecane (apolar solvent). This shows that this strain was electron donating. Conversely, for other pH values, no significant difference in affinity of cells surface to chloroform and hexadecane was observed, suggesting that no electron donor character was exhibited by this strain at these pH values. Results in Table 1 also showed that *E. coli* AL52 strain exhibited an electron acceptor character demonstrated by a greater affinity to diethyl ether (basic solvent) than to hexane from pH 5 to 9. For other pH no electron acceptor character was observed. The marked difference of electron donor/electron acceptor properties observed between the pH values could be attributed to the number and the type of dissociable groups such as carboxyl and phosphate exposed in the cell surface or to the distribution of these functional groups exposed on cellular structure. The finding presented here shows that *E. coli* strain studied here possess a strong electron donor and no electron acceptor at pH 2 and at neutral pH this strain has not exhibited the electron donor character. This later result is not consistent with studies[30,45], which have reported that the *E. coli* has a strong electron donor and a weak electron acceptor at neutral pH.

Glass surface characterization

The physico–chemical surface properties of glass surface were characterized by contact angle measurement. In brief, larger contact angle with polar probe liquid indicates that the surface is more hydrophobic. Conversely, large contact angle with apolar liquid indicates that the substrate is less hydrophobic (hydrophilic). As can be observed in Table 2, glass showed a $è_w = 35.7°$, indicating the relative hydrophilic character. Also glass surface exhibited high electron donor ($\gamma^- = 46$) and weak electron acceptor ($\gamma^+ = 1.14$).

Adhesion of *E. coli* to glass under different pH values

Fig. 1 shows the images of adhesion of *E. coli* to glass obtained by scanning electron microscopy. The cells deposited in cluster form

for pH 2 and pH 3. For other pH the adhesion was null or very low. In this study, the adhesion was quantified using Math lab program[38]. This program was used to analyse the images obtained by Scanning Electron Microscope (SEM), in order to determine the percentage of surface covered by cells of *E. coli*. This percentage was measured by the ratio of the covered surface and the total surface. The results are presented in Fig. 2. The adhesion of *E. coli* strain to glass was strongly influenced by pH with maximum adhesion occurring at pH 2. The percentage of

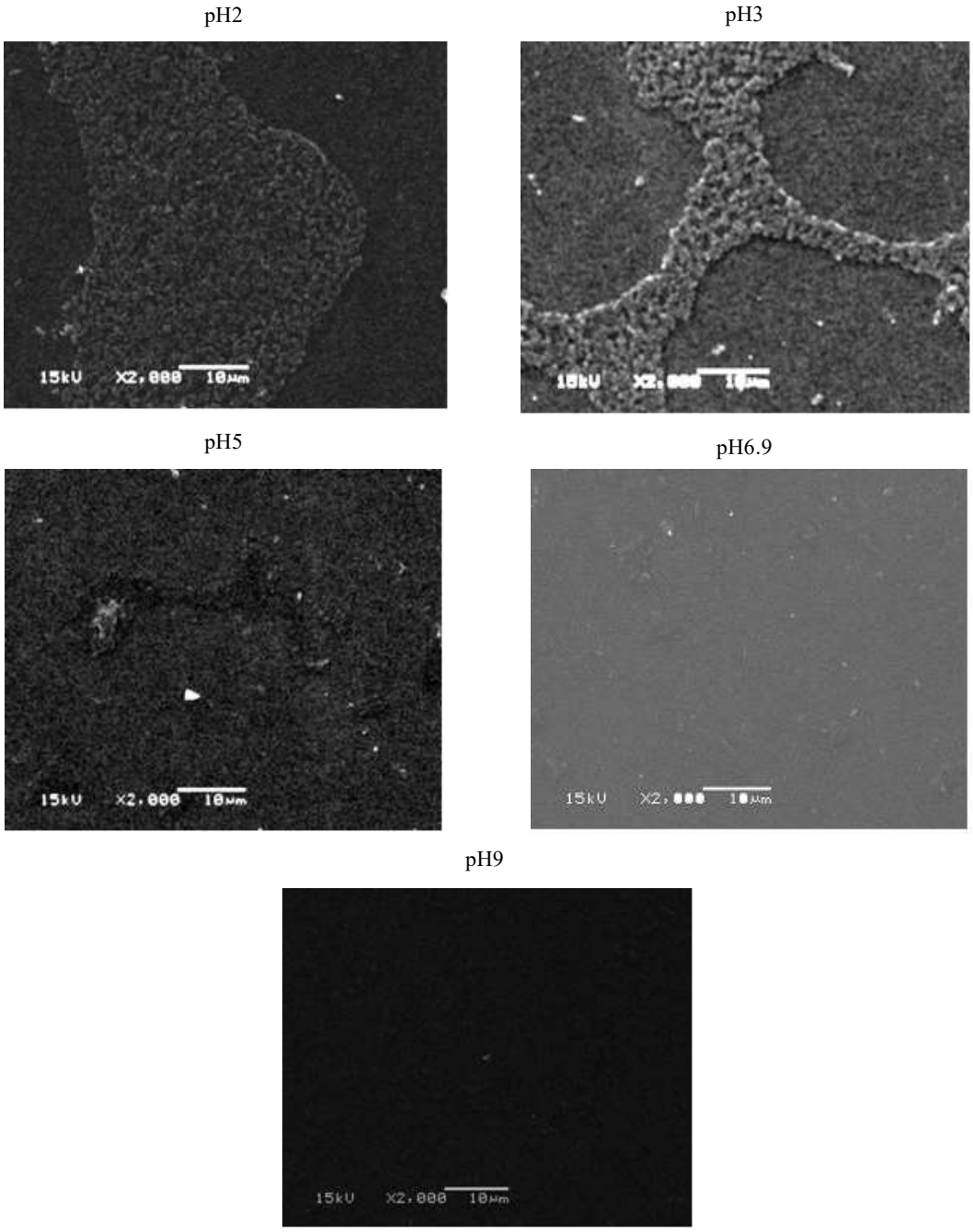

Fig. 1. SEM images of *E.coli* AL52 adhered to glass as a function of pH.

covered surface by cells decreased from 52% to 0% when pH varied from 2 to 9. It has been known that microbial adhesion to inert substratum surface originates from three fundamental forces, Lifshitz – Van der Waals, electrostatic and acid – base interactions. In this work, we did not investigate the role of electrostatic interactions because of its weak influence in media of high ionic strength used in this study. Consequently, in this work we focus to demonstrate mainly the role of electron donor character (acid-base interactions) in microbial adhesion. As can be seen in Fig. 1, the adhesion was maximal at pH 2. The cell surface was strong electron donating at pH 2 (Table 1) and glass was weak electron accepting (Table 2). According to Van Oss (1993)[18], the acid- base interactions were expressed between the protein with strong electron donor and the glass with discrete electron acceptor, so the maximum adhesion observed at pH 2 could be attributed to acid base interactions between *E. coli* with high electron donor character and glass surface with

Table 1. Hydrophobicity and electron donor/ electron acceptor of *Escherichia coli* AL52 as a function of pH. Results are the mean of three experiments.

pH	Affinity to chloroforom (%)	Affinity to hexadecane hydrophobicity (%)	Electron -donor character (%)	Affinity to diethyl-ether (%)	Affinity to hexane (%)	Electron acceptor character (%)
2	42.5 (0)	13 (1)	29.5	0 (0)	19 (1)	0
3	1 (1)	7 (0)	0	6 (4)	6 (0)	0
5	6 (4)	4 (4)	2	9 (1)	3 (3)	6
6.9	2 (2)	6 (4)	0	8 (2)	4 (0)	4
9	4 (2)	5 (4)	0	8 (2)	3 (1)	5

The standard deviation is given in parentheses

Table 2. Contact angle measurements of glass surface

Material	Contact angle θ (°)			Surface energy (mJ.m^{-2})		
	Water	Diiodomethane	Formamide	γ^{lw}	γ^{+}	γ^{-}
Glass	35.7	54.2	37	31.97	1.14	46.24

weak electron acceptor. The results presented here showed that the *E. coli* strain and glass were hydrophilic. So, the maximum adhesion obtained at pH 2 could be explained by the hypothesis which indicates that hydrophilic organism tend to attach to hydrophilic substratum. The maximal adhesion observed at pH 2 could be explained in the term of two groups of interactions: (i) acid – base interactions, (ii) hydrophilic –hydrophilic interactions.

This finding is consistent with the observations of some other works that used other bacteria[36-38] who found that, in addition to hydrophilic-hydrophilic interactions or hydrophobic–hydrophobic interactions, the

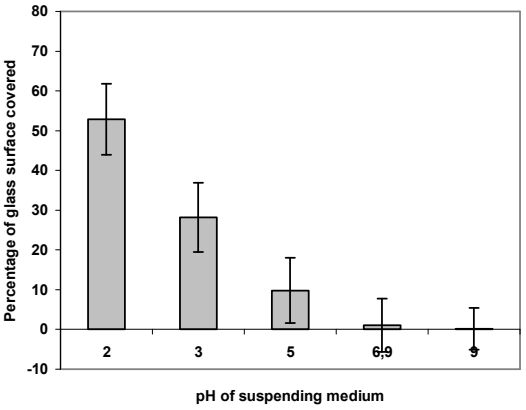

Fig. 2. Percentage of glass surface covered by cells of *Escherichia coli* AL52 as a function of pH

microbial adhesion could be mediated by the acid – base interactions.

For pH 3, the cell adheres to glass. Since the electron donor character was null at this pH, the adhesion could be due only to hydrophilic–hydrophilic interactions because both *E. coli* strain and glass were hydrophilic. For other pH the adhesion of *E. coli* to glass was very low or null. This result was surprising since hydrophilic cells are in general expected to adhere to hydrophilic substrata. Liu *et al.* (2004)[33] have proposed a model to understand the role of hydrophobic/hydrophilic interactions between cell and support surface in microbial adhesion, and using this model, they reported that microbial adhesion would proceed with difficulty if both bacterial and support surfaces were hydrophilic.

Others report[44] have examined the adhesion of *E. coli* to glass at high ionic strength (absence of repulsive electrostatic interactions) using Atomic Force microscopy (AFM). These authors found that *E. coli* with hydrophilic character does not adhere to glass with hydrophilic character. They reported that some other short-range physicochemical interactions such as cell surface structure might be responsible for the observed repulsive forces between *E. coli* and glass.

The results obtained in this work show that the hydrophilic–hydrophilic interactions seem to be playing a role in microbial adhesion but this role is not always sufficient to explain microbial adhesion between two hydrophilic surfaces.

Until now, most of the works have examined the role of hydrophobicity and electrostatic properties in the microbial adhesion process, but currently few information about the role of electron donor character in this process are available[18,38]. Results obtained here showed that the adhesion of *E. coli* strain was maximal when the electron donor character was very high. These indicate the crucial role of electron donor character in adhesion of the *E. coli* strain to glass. These results are consistent with our previous work[38] on *Staphylococcus aureus*, which found that the maximum adhesion to glass occurred when the electron donor character was very high.

CONCLUSIONS

In this study, we found that adhesion of *E. coli* to glass was influenced by pH of suspending medium. The adhesion was maximised when the electron donor character was very high indicating that this character plays a role in the adhesion of *E. coli* to glass. Results from this study also indicate that the type of interactions governing microbial adhesion depend on pH.

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