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EXPERIMENTAL ARTICLES

Evaluation of the Relative Cell Surface Charge by Using Microbial Adhesion to Hydrocarbon[1]

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Abstract—A simple and rapid method, Microbial adhesion to hexadecane, for estimating the cell surface charge is proposed. This method is based on the determination of cell affinity to hexadecane at low ionic strength and at high ionic strength. The difference between these two affinities can provide the relative cell surface charge. The application of this method for *Staphylococcus aureus* and *Escherichia coli* show that the profile of surface charge evolution as a function of pH was similar to these obtained by microelectrophoresis method.

Keywords: Relative cell surface charge, Microbial adhesion to hexadecane, *Staphylococcus aureus*, *Escherichia coli*.

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Microbial adhesion is the first in a series of events that occur during the colonization of a solid substratum. From an overall physic-chemical point of view, microbial adhesion can be mediated by non specific interactions including Lifshitz-van der Waals, electrostatic forces, and acid-base interactions. As soon as microorganisms reach a surface, they will be attracted or repelled by it depending on the sum of the different non specific interactions. These interactions are based on the cell surface charge [1, 2], the hydrophobicity [3] and the electron donor electron acceptor properties [4]. A knowledge of the physicochemical properties of a bacterium is important to predict the first step in adhesion process. Several methods were used to evaluate the cell surface properties and have been subject to comparison and discussion.

The microbial cell surface hydrophobicity is often evaluated by hydrophobic interaction chromatography [5], bacterial adhesion to hydrocarbon [6] and water contact angle measurement [7, 8] these methods have been subject to comparison [9–13]. Also the electron donor and electron acceptor properties have determined by several techniques including Potentiometric titration [14], Microbial adhesion to solvents [15] and contact angle measurement [7, 8]. These latter techniques have been compared and discussed extensively [13, 15].

Surface charge are among the parameters that affect bacterial adhesion and could be controlled this process. This surface charge originates from functional groups on the cell surface, including carboxylic phosphoric, hydroxyl and amine groups [16]. These functional groups are associated with peptidoglycan, teichoic acids on the surface of gram-positive bacteria and with lipopolysaccharides, phospholipids and proteins on the surface of gram negative bacteria. Several experimental techniques have been used to determine cell surface charge including, electrostatic interaction chromatography [17] aqueous two-phase partitioning [18–20] acid-base titration [14, 21], and microelectrophoresis [22, 23]. Up till now, there is no noticeable debate on the efficacy of these methods because the microelectrophoresis, in which the electrophoretic mobility (EPM) of charged particles is measured in an externally applied electric field, is considered to be the common method used to determine cell surface charge. This method requires relatively elaborate and cost equipment. Thus to facilitate the characterization

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of cell surface charge we have developed a simple, rapid technique, Microbial adhesion to hexadecane (MATH) which was proposed by Rosenberg et al. 1980 [6] to determine the cell surface hydrophobicity.

The aim of this paper is to use the MATH method to evaluate the relative cell surface charge of two strains *Staphylococcus aureus* ATCC 25923 and *E. coli* AL52.

MATERIALS AND METHODS

Bacterial strains and growth conditions. 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 *g*, washed twice and resuspended in the suspending liquid (KNO_3, 10^{-3} or 10^{-1} M).

Microbial adhesion to hydrocarbon. This method previously described by Rosenberg et al. 1980 [6] is based on the estimation of cell adherence to the hydrocarbon water interphase in a biphasic system containing hexadecane, n-octane or *p*-xylene to evaluate qualitatively the cell surface hydrophobicity. In this work we have used this method to estimate the cell surface charge. In this context, we have determined the cell affinity to hexadecane at different pH and under two ionic strength 10^{-3} and 10^{-1} [24]. 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 affinity for hexadecane 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.

Estimation of cell surface charge at different pH using MATH. It is known that the hydrocarbons in suspension are hydrophobic and negatively charged [25–27]. However, the MATH measures a complicated interplay of hydrophobic and electrostatic interactions [28, 29]. In the other hand, at high electrolyte concentration the electrostatic contribution is neglected. Thus the cell affinity to hexadecane at high ionic strength and low ionic strength could presented in the flowing formula respectively

At low ionic strength (10^{-3}):

Affinity to hexadecane = electrostatic interactions + van der waals interactions (1);

At high ionic strength (10^{-1}):

Affinity to hexadecane = van der waals interactions (2).

Based on both formulas (1) and (2) the surface charge at (10^{-3}) could be estimated by the difference between affinity to hexadecane at low ionic strength (10^{-3}) and affinity to hexadecane at high ionic strength (10^{-1}).

RESULTS AND DISCUSSION

As reported previously by some works, the affinity to hexadecane reflect electrostatic interactions and van der waals interactions at low ionic strength and only van der waals interactions at high ionic strength. In this context, we have proposed that the surface charge is the result of the difference between these two affinities to hexadecane. The value of this relative charge estimated for *E. coli* and *S. aureus* are presented in Tables 1 and 2 respectively. The value of surface charge is positive at pH 2, pH 3 for *S. aureus* and at pH 3 for *E. coli*. This value is negative for other pH

Table 1. Relative surface charge of *S. aureus* estimated using microbial adhesion to hexadecane

pH	Affinity to hexadecane at two ionic strength, %		Relative surface charge, %
	0.001 M	0.1 M	0.001 M
2	95 (3)	72 (14)	23
3	76 (18)	63 (32)	13
5	22 (2)	42 (9)	–20
6.2	4 (2)	29 (9)	–25
9	1 (1)	39 (8)	–38
11	17 (6)	75 (13)	–58

Note: standard deviation was given in parentheses.

Table 2. Relative surface charge of *E. coli* estimated using microbial adhesion to hexadecane

pH	Affinity to hexadecane at two ionic strength, %		Relative surface charge, %
	0.001 M	0.1 M	0.001 M
2	26 (1)	13 (1)	13
3	3 (3)	7 (0)	–4
5	1 (1)	4 (4)	–3
6.5	0 (0)	6 (4)	–6
9	0 (0)	5 (4)	–5
11	0 (0)	3 (3)	–3

Note: standard deviation was given in parentheses.

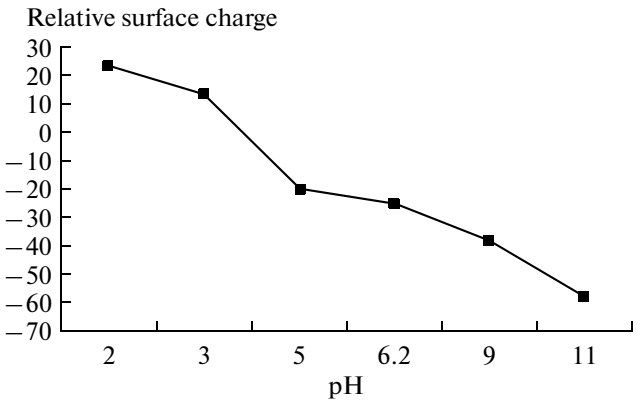

Fig. 1. Evolution of *S. aureus* reltive surface charge as a function of pH estimated by MATH.

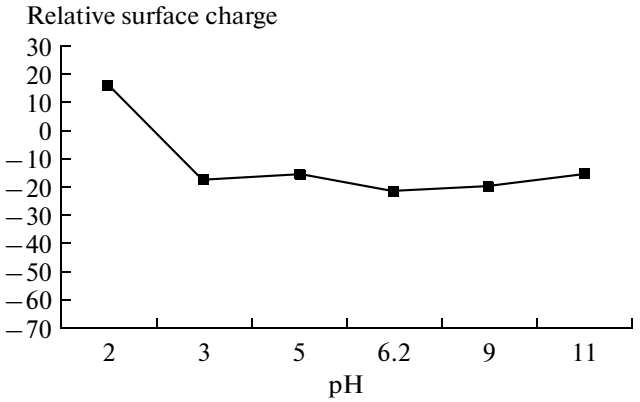

Fig. 2. Evolution of *E. coli* relative surface charge as a function of pH estimated by MATH.

for *S. aureus* and *E. coli*. The evolution of this charge of both strains is presented in Figs. 1 and 2. As shown in these figures, *S. aureus* seem to be more charged that *E. coli*. This difference could be attributed to difference in cell surface chemical composition. Several works [16, 23, 30] have reported that a phosphate group plays the major role in determining the surface electrostatic charge by examining the correlation between electrostatic charge and the surface chemical composition. The P/C ratio of Staphylococci varied from 0.02 to 0.04 [31] and of *E. coli* varied from 0.009 to 0.02 [22, 23]. This indicates that the phosphate groups are higher on the Staphylococci cell surface than the *E. coli* cell surface which explains the high negative charge of *S. aureus* than *E. coli*. Dickson and Koohamaraie (1998) [1] have used the electrostatic interaction chromatography method to determine the cell surface charge and they reported that *S. aureus* was more negative than *E. coli* at neutral pH. These finding are similar to results obtained here. Moreover, the profile of surface charge as a function of pH estimated by MATH are very similar to those obtained with microelectrophoresis method [23, 32]. Finally, we conclude that microbial adhesion to hexadecane could be used as method to estimate the surface charge of bacteria.

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