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Colloids and Surfaces B: Biointerfaces 44 (2005) 41–48
COLLOIDS AND SURFACES B
www.elsevier.com/locate/colsurfb

Bacterial adhesion to silica sand as related to Gibbs energy variations

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Received 8 July 2004; received in revised form 16 May 2005; accepted 16 May 2005

Abstract

Bacterial adhesion to silica sand was related to variations in system Gibbs energy ΔG_{adh}. Two typical Gram-positive bacterial strains of *Streptococcus mitis* and *Lactobacillus casei* were used as the model bacteria in this research. Impacts of solution chemistry and goethite coating of silica sand on bacterial adhesion were also explored. *S. mitis* and *L. casei* had negative ΔG_{adh} with both uncoated and goethite-coated silica sand, demonstrating their adhesion potentials to these substrate. After goethite coating, ΔG_{adh} decreased (negatively increased) for both *S. mitis* and *L. casei*. In the presence of rhamnolipid biosurfactant, ΔG_{adh} increased (negatively decreased) in answer to the increase of the rhamnolipid biosurfactant concentration. Bacterial percentage adhesion to silica sand corresponded to ΔG_{adh}. This study demonstrated that bacterial adhesion to substrate could be explained in terms of bacterial, substratum and intervening medium physicochemical surface properties, which can be independently determined based on contact angle measurements.

Keywords: *Streptococcus mitis*; *Lactobacillus casei*; Silica sand; Goethite; Rhamnolipid biosurfactant

1. Introduction

Bacterial adhesion is important in a variety of environmental applications including microbial biofouling and in situ bioremediation. When conditions permit, attached bacterial cells may survive for prolonged periods and biofilms, i.e., immobilized bacterial cell colonization on a surface, can be formed. Bacterial adhesion and surface colonization are correlated with bacterial surface physicochemical properties [1–4], which ascribes to the surface molecular composition in terms of proteins, polysaccharides and hydrocarbon-like compounds [5]. Bacterial strains with different cell surface properties show different adhesion kinetics and affinity for substrate [6]. Bacterial surface physicochemical properties can be chemically modified to stimulate or impede bacterial adhesion [7,8]. Also, several extracellular structures, such as lipopolysaccharides, flagella and membrane proteins may impact the modulation of the adhesion of bacteria to substrate [9–11].

Bacterial adhesion begins with long-range, non-specific interactions between bacterial cells and substrate, which are unstable and bacterial cells at this stage, can be removed from adsorbed surfaces by fluid shear before firm adhesion can occur. Once bacterial cells are in close proximity to a surface, they can establish short-range, specific interactions, which are stable and are able to glue bacterial cells to the surface. These two processes together refer to initial adhesion, which is dependent upon physicochemical properties of bacterial cell and substratum surfaces, as well as the intervening medium. It has been proven that the initial adhesion of bacteria to substrate plays an important role in bacterial adhesion [12–14]. It has also been proposed that bacterial adhesion to abiotic surfaces leads to variations in system Gibbs energy [15], such that,

$$\text{Bacteria (aqeous phase)} + \text{Substrate (solid)} \Leftrightarrow \text{Substrate with adhered bacteria (solid)}\ \Delta G_{adh} \quad (1)$$

where ΔG_{adh} is the Gibbs energy change when bacteria adsorb to substrate from the aqueous phase (mJ). According to the traditional and extended Derjaguin–Landau–Verwey–

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0927-7765/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.colsurfb.2005.05.012

Overbeek (DLVO) theory, bacterial and substratum physicochemical properties can be described in terms of surface thermodynamic parameters of van der Waals component of surface tension (γ^{LW}), electron-acceptor parameter (γ^{+}) and electron-donor parameter (γ^{-}) of Lewis acid/base component of surface tension and ζ-potential, which can be determined independently. Thus, ΔG_{adh} can be calculated based on independently determined surface thermodynamic properties of bacteria, substrate and the intervening medium.

The objective of this research is to assess bacterial adhesion to silica sand as related to variations in system Gibbs energy. Two typical Gram-positive bacterial strains of *Streptococcus mitis* and *Lactobacillus casei* were used as the model bacteria. Impact of solution chemistry on bacterial adhesion was explored in the presence of rhamnolipid biosurfactant, which was produced by *Pseudomonas aeruginosa* during the late logarithmic growth phase. Also, effect of substratum surface properties on bacterial adhesion was investigated by means of goethite coating. It is the ultimate goal of this research to demonstrate that bacterial adhesion can be explained in terms of their interaction free energies with the substrate, which are determined by the surface properties of bacteria, substrate and the intervening medium.

2. Materials

2.1. Bacterial strains

Bacterial strains used in this research were typical Gram-positive strains of *S. mitis* (ATCC 9456) and *L. casei* (ATCC 11578). *S. mitis* was cultured in Todd Hewitt broth (THB) and *L. casei* was cultured in De Man, Rogosa and Sharpe broth (MRS). Both bacterial strains were quantified using Adenosine Triphosphate (ATP) analysis [3]. Briefly, inoculated bacteria were cultured on a Gyrotory Water Bath Shaker (New Brunswick Scientific Co. Inc., Model G76). Fifty micrometer culture was sampled regularly and reacted with luciferase. The number of viable bacterial cells was then obtained based on the light emitted by the reaction of ATP extracted from cells with luciferase, as measured by a luminometer (Turner Design, TD-20/20). ζ-Potentials of these two bacterial strains were determined from their electrophoretic mobility based on the Smoluchowski equation [16]:

$$\zeta = \frac{\delta\eta}{\varepsilon\varepsilon_0} \quad (2)$$

where ζ is the zeta potential measured at the slipping plane (V), δ is the electrophoretic mobility [(m/s)/(V/m)], η is the dynamic viscosity of the solution (N/(s m^2)) and ε and ε_0 are the relative dielectric permittivity of water (78.55 for water at 25 °C) and permittivity under vacuum [8.854×10^{-12} C/(V m)], respectively. Electrophoretic mobility of the bacterial strains was measured in a pH 7.0 phosphate buffer solution (0.04 M potassium phosphate monobasic–sodium hydroxide buffer, diluted with distilled water 1:25 (v/v), Fisher Scientific, Pittsburgh, PA) by dynamic light scattering (Zetasizer 3000HAS, Malvern Instruments Ltd., Malvern, UK) as described by Meinders et al. [15].

2.2. Silica sand

Silica sand (Fisher Scientific, eight mesh) used in this research was first rinsed using de-ionized water and then treated with sodium acetate, sodium dithionate and sodium citrate to remove iron ions and hydrogen peroxide to remove organic matters. The silica sand was then saturated with Na^+ using 1 M phosphate-buffered saline (pH 7.0). Before experiments, the silica sand was sterilized and stabilized by extensive washing with sterilized de-ionized water until the electrical conductivity was less than 1 μS/cm. Silica sand specific surface areas were measured by a surface area analyzer using krypton adsorption isotherms (ASAP 2010, Micromeritics, Norcross, GA). ζ-Potentials of silica sand were measured on particles obtained by grinding, suspended in the pH 7 phosphate buffer solution.

2.3. Goethite coating

Goethite was prepared as described by Schwertmann et al. [17] and Peak et al. [18]. Briefly, 1.0 M ferric nitrate was mixed with 1.0 M KOH (1:9, v/v) and aged for 21 days at 25 °C. This suspension was then washed extensively with de-ionized water via centrifugation. The rinsed solid was resuspended in 0.4 M HCl. After washed and dialyzed against de-ionized water, it was freeze dried to obtain crystalline goethite. Thus, obtained goethite was then coated on silica sand following the method of Schwertmann et al. [17] and Scheidegger et al. [19]. Briefly, goethite were mixed with silica sand (1:5, w/w) in 0.01 M $NaNO_3$ solution (pH 7.5) and shaken for 48 h. Coated silica sand was then washed with 0.1 M $NaNO_3$ (pH 7.0) via centrifugation. After rinsed with de-ionized water, coated silica sand was oven-dried at 110 °C. Goethite coating was determined by dissolving coated silica sand in HNO_3 (95%) and HF (40%) (2:1, v/v). Specific surface areas of goethite-coated silica sand were also measured by the surface area analyzer.

2.4. Rhamnolipid biosurfactant production and extraction

After inoculated with 1 ml (1.0%) stationary phase culture, *P. aeruginosa* (ATCC 9027) was grown in Kay's minimal medium, which was composed of 0.3 g $NH_4H_2PO_4$, 0.2 g K_2HPO_4, 0.2 g glucose, 0.5 mg $FeSO_4$ and 0.1 g $MgSO_4{\cdot}7H_2O$ at 37 °C for 24 h. Two millilitre of above culture was used to inoculate 200 ml of phosphate-limited proteose peptone–glucose–ammonium salt medium, which consisted of 1.0 g NH_4Cl, 1.5 g KCl, 19.0 g Tris–HCl, 5 g glucose, 1 g proteose peptone and 0.4 g $MgSO_4{\cdot}7H_2O$, adjusted