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Serum as a Factor Influencing Adhesion of *Enterococcus faecalis* to Glass and Silicone

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The purpose of this work was to analyze the effect of serum on the physicochemical surface properties and adhesion to glass and silicone of *Enterococcus faecalis* ATCC 29212 at 37°C. As is presented using thermodynamics analysis, serum minimizes the interaction of cells with water, which correlates well with the increase in hydrophobicity and in bacterial adhesion to glass and silicone.

Enterococci are important nosocomial pathogens (7, 10), with *Enterococcus faecalis* accounting for 90% of human enterococcal infections, including bacteremia, wound infections, abdominal and urinary tract infections, and endocarditis (18, 22). Bacterial adhesion to biomaterials (14, 21) is directly responsible for increases in bacterial multiplication and biofilm formation (1). Despite the fact that knowledge of enterococcal virulence mechanisms is still limited (9, 18), it is generally agreed that before bacteria anchor to the host surface, the physicochemical characteristics of both the surface cell and the substratum are the main factors mediating bacterial adhesion (4, 16, 20, 23, 24). In this context, the cellular surface hydrophobicity is, for some authors, the most important physicochemical parameter controlling adhesion (1, 12, 15). The initial adhesion step in high-ionic-strength medium (17) can be also interpreted in terms of the Lifshitz-van der Waals forces (LW) and acid-base forces (AB) (4, 15, 20).

Because physicochemical characterization depends on cell surface properties and these in turn depend on the culture and experimental conditions, this paper attempts to analyze the influence of the culture medium on the adhesion of *E. faecalis* ATCC 29212 to glass and silicone rubber at a temperature similar to that inside the human body (37°C). The surface characterization and adhesion experiments on both substrata were carried out in a parallel plate flow chamber with bacteria grown in a standard culture medium and in the same medium supplemented with 10% human serum.

Microorganisms were stored in porous beads at −80°C; blood agar plates were inoculated with *E. faecalis* ATCC 29212 at 37°C and then incubated overnight in 100 ml of Trypticase soy broth (TSB), without or with 10% human serum. The cells were harvested by centrifugation for 5 min at 1,000 × *g* and washed three times with phosphate-buffered saline for flow experiments (final concentration, 3×10^8 cells ml^{-1}) and with distilled and deionized water for contact angle assays.

Glass was acid cleaned, and silicone (kindly provided by Willy Rüsch AG, Stuttgart, Germany) was cleaned by sonication with an available surfactant solution. Both were extensively washed with water.

The parallel plate flow chamber has been described in detail previously (19). A parallel plate flow chamber was placed on the stage of a microscope equipped with a 40× ultra-long-working-distance objective. Bacterial suspensions were recirculated with a pulse-free flow of 0.034 ml s^{-1} while the system was maintained at 37°C. Images of microorganisms adhering to the bottom plate of the flow chamber were registered by a charge-coupled device camera and stored in a computer. The images were captured every 2 min at the beginning of the adhesion process and, after that, at every 10 min of the adhesion process up to 4 h (data presented in Fig. 1; also see Table 3).

Experiments were done in triplicate and compared with an unpaired Student *t* test (confidence interval, 95%).

Using the sessile drop technique (2), water, formamide, and diiodomethane contact angles (θ_W, θ_F, and θ_D, respectively) were determined on lawns of dried bacteria (Table 1). Briefly, bacteria suspended in demineralized water were layered onto 0.45-μm-pore-size filters (Millipore, Molsheim, France) by using negative pressure. Filters were left to air dry for 45 min at 37°C and were then placed in an environmental chamber, which was connected to a thermostat to maintain the temperature at 37°C and saturated with vapor of the liquid used. The images were taken as has been previously described (13).

The surface tension components (Table 1), γ^{LW} and $\gamma^{AB} = \sqrt{\gamma^+ \cdot \gamma^-}$, were calculated from the application of the Young-Dupré equation (25) to each probe liquid:

$$\gamma_L(\cos\theta_L + 1) = 2\sqrt{\gamma_B^{LW} \cdot \gamma_L^{LW}} + 2\sqrt{\gamma_B^+ \cdot \gamma_L^-} + 2\sqrt{\gamma_B^- \cdot \gamma_L^+} \qquad (1)$$

where γ^- and γ^+ are the electron donor and electron acceptor parameters of the γ^{AB} component, respectively, $\gamma_L^{LW} + \gamma_L^{AB}$ is the surface tension of the probe liquid (*L*), and *B* is bacteria.

Components and parameters of surface tension of probe liquids at 37°C were calculated according to González-Martín et al. (5).

According to van Oss et al. (25), the total interaction free energy between microorganisms and substrata through water (ΔG_{adh}^{Total}) can be calculated as the sum of a term which takes into account the interaction between them through dipole-dipole, dipole-induced dipole, and induced dipole-induced dipole LW interactions and a second term which takes into

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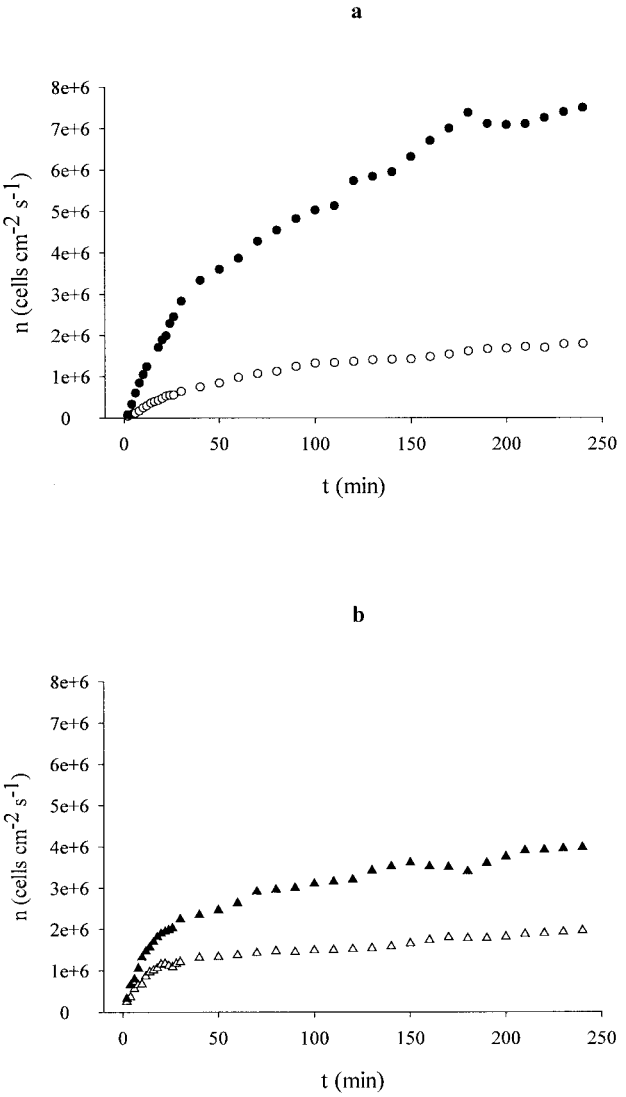

FIG. 1. (a) Average levels of adhesion to glass of *Enterococcus faecalis* ATCC 29212 grown in serum-free medium (○) and 10% serum-containing medium (●) during the duration of the experiment. The triplicate experiments coincided within a 15% margin of error. (b) Average levels of adhesion to silicone of *Enterococcus faecalis* ATCC 29212 grown in serum-free medium (△) and 10% serum-containing medium (▲) during the duration of the experiment. The triplicate experiments coincided within a 15% margin of error.

account their tendency to give or accept electrons, which is their Lewis or acid-base character:

$$\Delta G_{adh}^{Total} = \Delta G_{adh}^{LW} + \Delta G_{adh}^{AB} \quad (2)$$

where

$$\Delta G_{adh}^{LW} = (\sqrt{\gamma_B^{LW}} - \sqrt{\gamma_S^{LW}})^2 - (\sqrt{\gamma_B^{LW}} - \sqrt{\gamma_W^{LW}})^2 - (\sqrt{\gamma_S^{LW}} - \sqrt{\gamma_W^{LW}})^2 \quad (3)$$

and

$$\Delta G_{adh}^{AB} = 2[\sqrt{\gamma_W^+}(\sqrt{\gamma_B^-} + \sqrt{\gamma_S^-} + \sqrt{\gamma_W^-}) + \sqrt{\gamma_W^-}(\sqrt{\gamma_B^+} + \sqrt{\gamma_S^+} + \sqrt{\gamma_W^+}) - \sqrt{\gamma_B^-\gamma_S^+} - \sqrt{\gamma_B^+\gamma_S^-}] \quad (4)$$

TABLE 1. Water, formamide, and diiodomethane contact angles of *Enterococcus faecalis* ATCC 29212 grown in serum-free TSB and 10% serum-containing TSB, as well as contact angles for both employed substrata, and Lifshitz-van der Waals (γ^{LW}), acid-base (γ^{AB}), and total (γ^{Total}) surface tension component and electron donor (γ^-) and electron acceptor (γ^+) parameters of microorganisms and employed substrata[a]

Growth medium or substratum	Contact angle (degrees)			Parameter value (mJ m^{-2})				
	θ_W	θ_F	θ_D	γ^{LW}	γ^-	γ^+	γ^{AB}	γ^{Total}
Growth media								
TSB	49	37	47	27.0	28.2	2.7	17.4	44.4
TSB + 10% serum	68	36	47	30.3	7.2	4.1	10.9	41.2
Substrata								
Glass	19	16	30	31.5	50.1	2.3	21.5	53.0
Silicone	92	89	67	19.4	11.7	0.4	4.3	23.7

[a] The average standard deviation over three separate cultures of microorganisms came to ±2 degrees for contact angles, ±2.0 mJ m^{-2} for γ^{LW}, ±5.0 mJ m^{-2} for γ^-, and ±0.8 mJ m^{-2} for γ^+.

and *W* is water and *S* is the substratum. Results are summarized in Table 2. Serum-grown bacteria present higher hydrophobicity than serum-free-grown cells, considering θ_W as an indicator of hydrophobicity (2). This behavior is in agreement with that found by Ljungh and Wadstrom, even working with a different methodology (11), although some other authors detected decreases or no changes in surface hydrophobicity after growth in serum (3, 6).

Comparing the hydrophobicity results with those of adhesion experiments, the highest hydrophobicity is well correlated with the greatest adhesion to both substrata, as presented in Fig. 1 and Table 3 ($P < 0.05$ for comparisons between serum-grown and serum-free-grown bacteria at the beginning of and after 4 h of the adhesion experiment). A similar agreement is seen between thermodynamics predictions and adhesion data: despite the positive value of ΔG_{adh}^{Total} for the adhesion to glass of serum-free-grown bacteria, thermodynamics calculations clearly predict that serum would be able to increase the adhesion to glass and silicone. This behavior is mainly due to the changes in the short-range ABs, because the long-range LWs remain unchanged under the different growth medium conditions.

A different approach to the information that the free energy values for total adhesion provide can be obtained by taking

TABLE 2. Lifshitz-van der Waals (ΔG_{adh}^{LW}), acid-base (ΔG_{adh}^{AB}), and total (ΔG_{adh}^{Total}) interaction free energy levels of the adhesion of *Enterococcus faecalis* ATCC 29212 grown with and without serum to glass and silicone in water

Growth medium	Substratum	Interaction free energy value (mJ m^{-2})		
		ΔG_{adh}^{LW}	ΔG_{adh}^{AB}	ΔG_{adh}^{Total}
TSB	Glass	−1.5	16.1	14.6
TSB	Silicone	0.1	−7.9	−7.8
TSB + 10% serum	Glass	−2.2	−3.8	−6.0
TSB + 10% serum	Silicone	0.2	−29.6	−29.4

The average standard deviation over three separate cultures of microorganisms was ±0.5 mJ m^{-2} for ΔG_{adh}^{LW} and ±5 mJ m^{-2} for ΔG_{adh}^{AB}.

TABLE 3. Average number of *Enterococcus faecalis* ATCC 29212 bacteria adhering to glass and to silicone initially (j_0 and at 4 h from the beginning of the adhesion experiments, taking into account the microorganism growth with or without serum

Growth medium	Substratum	j_0[a]	No. of adhered bacteria (10^6)
TSB	Glass	383	1.8
TSB	Silicone	565	2.0
TSB + 10% serum	Glass	1,562	7.5
TSB + 10% serum	Silicone	1,483	4.0

[a] j_0, initial adhesion rates calculated from Fig. 1.

into account that $\Delta G_{adh}^{\text{Total}}$ represents the total free energy interaction between medium 1 (bacteria) and medium 2 (substratum) when immersed in a given medium 3 (water) (expressed as $\Delta G_{1,3,2}$). Using the method described in reference (8), this can be expressed as

$$\Delta G_{1,3,2} = \Delta G_{1,2} - \Delta G_{1,3} - \Delta G_{2,3} - 2\gamma_3 \quad (5)$$

where $\Delta G_{1,2}$, $\Delta G_{1,3}$, and $\Delta G_{2,3}$ are the interaction free energy values (in a vacuum) between media 1 and 2, 1 and 3, and 2 and 3, respectively, and γ_3 is the surface tension of medium 3. The different ΔG_{ij} values, where i and j symbolize 1, 2, or 3, have been calculated (Table 4) based on the fact that

$$\Delta G_{ij} = \gamma_{ij} - \gamma_i - \gamma_j = -2(\sqrt{\gamma_i^{\text{LW}} \cdot \gamma_j^{\text{LW}}} + \sqrt{\gamma_i^{+} \cdot \gamma_j^{-}} + \sqrt{\gamma_i^{-} \cdot \gamma_j^{+}}) \quad (6)$$

This way of splitting the total free energy permits a deeper understanding of the forces that provoke the increase in adhesion of serum-grown bacteria to both substrata.

From the data presented in Table 4, it can be clearly seen that addition of serum to the growth medium does not affect the direct interaction between bacteria and either glass or silicone rubber, the two substrata. However, there was an important change of about 20 mJ m^{-2} between the ΔG_{BW} values before and after serum growth. Taking this fact into account, the highest adhesion observed for serum-grown bacteria, regardless of the substratum selected, can be seen to be more closely related to changes in their behavior with water than to the kind of substratum used ([ΔG_{BW} (with serum)] < [ΔG_{BW} (without serum)]). The lower affinity of serum-grown bacteria for water drives the cells to more favorable surroundings (i.e., with regard to substrata).

It is interesting that the data obtained from the splitting of $\Delta G_{adh}^{\text{Total}}$, although in accordance with that obtained from the measurement of water contact angles (i.e., higher hydrophobicity for serum-grown cells than for serum-free-grown bacteria) provides a step further towards the analysis of the adhesion behavior of this bacterium, since it provides a possible elucidation of how hydrophobicity changes act on the adhesion process.

TABLE 4. ΔG_{12} interaction free energies for *Enterococcus faecalis* ATCC 29212 bacteria (*B*), grown without serum and with serum, with glass (*G*), silicone rubber (*R*), and water (*W*)[a]

Growth medium	Interaction free energy value (mJ m^{-2}) with:		
	ΔG_{BG}	ΔG_{BR}	ΔG_{BW}
TSB	−98	−64	−117
TSB + 10% serum	−97	−66	−97

[a] ΔG_{12} interaction free energy value between silicone rubber and water is $\Delta G_{RW} = -81$ mJ m^{-2} and between glass and water is $\Delta G_{GW} = -137$ mJ m^{-2}. The average standard deviation over three separate cultures of microorganisms was 5 mJ m^{-2}.

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