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Original research paper

The role of microbial surface properties and extracellular polymer in *Lactococcus Lactis* aggregation

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ABSTRACT

Microbial adhesion to an interface is known to have an important role in a wide variety of situations. In this study, we examine the effect of the surface physicochemical properties and extracellular polymers (ECP) of a lactic bacterium on microbial aggregation. *Lactococcus lactis* JCM 5805 was used in the current experiments to investigate the factors that control microbial aggregation. To this aim, we measured the electrophoretic mobility and contact angle of *L. lactis*. As a result, *L. lactis* was found to be a negatively charged and hydrophilic bacterium. The microbial aggregation was investigated using DLVO (Derjaguin, Landau, Verwey and Overbeek) theory. The percent aggregates of washed cells increased slightly with increasing ionic strength of the cell suspension. This tendency agreed with the prediction of the DLVO theory. By contrast, when the ECP was present in suspensions of intact cells or washed cells, the ECP was found to promote the aggregation of the microbial cells in the low ionic strength region due to an attractive bridging force. The percent aggregates of microbial cell decreased with increasing the ionic strength due to the repulsive steric or overlap forces between polymer-covered surfaces.

1. Introduction

Microbial adhesion to an interface is known to have an important role in a wide variety of situations. Microbial adhesion is beneficial in applications such as fermentation, wastewater treatment, bioremediation, and bioleaching [1–4]. In contrast, microbial adhesion causes problems in food contamination, medical implant and device infection, ship fouling, metallic corrosion, and dental caries [5–9]. Microbial adhesion can occur on any surface exposed to an aqueous environment. Despite this, a detailed mechanism for microbial adhesion has not yet been described. If the adhesion mechanism employed by microorganisms can be understood, it will allow the utilization or control adherent microorganisms. In this paper, we consider a microorganism to be a "living particle", and use the techniques of fine particle technology to understand the mechanism of microbial adhesion.

The aim of this study is to examine the effect of the surface physicochemical properties of a lactic bacterium on microbial aggregation. Microbial aggregation has been employed in the upflow anaerobic sludge blanked (UASB) reactor. Several models for the models of microbial aggregation have been proposed [10]. However the mechanisms have not yet been clarified. In addition, we also examine the effect of extracellular polymers (ECP) metabolized by microorganisms as one of the factors of microbial aggregation. *Lactococcus lactis* JCM 5805 was used to investigate the factors that control microbial aggregation. The surface potential and surface tension of microbial cells were estimated, and the microbial aggregation was investigated using the traditional DLVO (Derjaguin, Landau, Verwey and Overbeek) theory and comparing predicted with actual values.

2. Materials and methods

2.1. Strain and growth conditions

L. lactis JCM 5805 was purchased from the Japan Collection of Microorganisms. *L. lactis* is a nonmotile micrococcus without flagella. *L. lactis* was grown aerobically at 30 °C in MRS (de Man-Rogosa-Sharpe) medium with agitation. Cells were harvested in the late exponential growth phase by centrifugation at 10,000 rev/min (8400*g*) at 4 °C for 10 min. Two types of cells were prepared, washed cells – washed three times using physiological saline to remove ECP and intact unwashed cells. The prepared cells were re-suspended in sterile NaCl aqueous solution at the desired concentration (pH 7.0). The surface physicochemical properties were then evaluated. All measurements described below were carried out in triplicate.

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Nomenclature

a	radius of particle (m)
A	Hamaker constant (J)
d	separation distance between surfaces (m)
d_0	minimum equilibrium distance (m)
e	electron charge (C)
F	percent aggregates of microbial cell (%)
k	Boltzmann constant (J/K)
N	density of charged group within the polymer layer ($1/m^3$)
OD_0	initial optical density of cell suspension before agitation (–)
OD_t	optical density of cell suspension after agitation (–)
u	electrophoretic mobility ($m^2/(Vs)$)
V_A	attractive van der Waals interaction energy (J/m^2)
V_R	repulsive electrostatic interaction energy (J/m^2)
Z	valency of charged groups within the polymer layer (–)
ΔG_{adh}	free energy of interaction between microbial cells (J/m^2)
ε	permittivity of medium (F/m)
γ	surface tension (J/m^2)
γ^+	electron-acceptor parameter of surface tension (J/m^2)
γ^-	electron-donor parameter of surface tension (J/m^2)
η	viscosity of medium (Pas)
κ	Debye–Hückel reciprocal length (1/m)
κ_m	Debye–Hückel parameter of the polymer layer (1/m)
λ	softness parameter, which has dimensions of reciprocal length (m)
θ	contact angle (deg)
Ψ	outer plane potential (V)
Ψ_0	potential at the boundary between the surface region and the solution (V)
Ψ_{DON}	Donnan potential of the polymer layer (V)

Subscripts

B	bacterium
L	liquid
BL	bacteria–liquid
LW	Lifshitz-van der Waals component
EL	electrostatic component
AB	Lewis acid–base component

2.2. Characterization

The electrophoretic mobility (EPM) of microbial cells was measured using an electrophoretic light-scattering spectrophotometer (ELS-800, Otsuka Electronics). The surface potentials were calculated using soft particle theory [11]. The apparent advancing contact angle of the microorganisms was approximated from measurements between a layer of microbial cells and a droplet of a specified fluid using a contact angle analyzer (FTA125, First Ten Ångstroms) [12]. Surface tensions were calculated using the Young-Dupré equation [13]. The polar and non-polar solvents used as probe liquids are shown in Table 1.

The size distribution of the microbial cells was measured using a particle size analyzer (NICOMP 380/ZLS, Particle Sizing Systems). The median diameter and the geometric standard deviation of *L. lactis* were 0.95 μm and 1.29 μm, respectively. The morphology of the microbial cells was observed using a field emission scanning electron microscope (FE-SEM) (JSM-6700F, JEOL) in high-vacuum mode at 10 kV. The subject cells were fixed with glutaraldehyde to observe their morphology by FE-SEM by techniques that we have developed specifically for this purpose [14]. A representative FE-SEM image of *L. lactis* is shown in Fig. 1.

2.3. Analysis of ECP

The contents of total carbohydrate and uronic acid contained in the ECP were measured by both the phenol–sulfuric acid method [15] and the carbazole-sulfuric acid method [16] with D-galacturonic acid as a standard. The content of protein was estimated by the Bradford method with bovine serum albumin as a standard [17].

Table 1
Polar and non-polar solvents used as probe liquids.

Probe liquid	Surface tension (mJ/m^2)				
	γ^{LW}	γ^+	γ^-	γ^{AB}	γ^{Total}
Water	21.8	25.5	25.5	51.0	72.8
Formamide	39.0	2.28	39.6	19.0	58.0
α-Bromonaphtalene	44.4	0	0	0	44.4

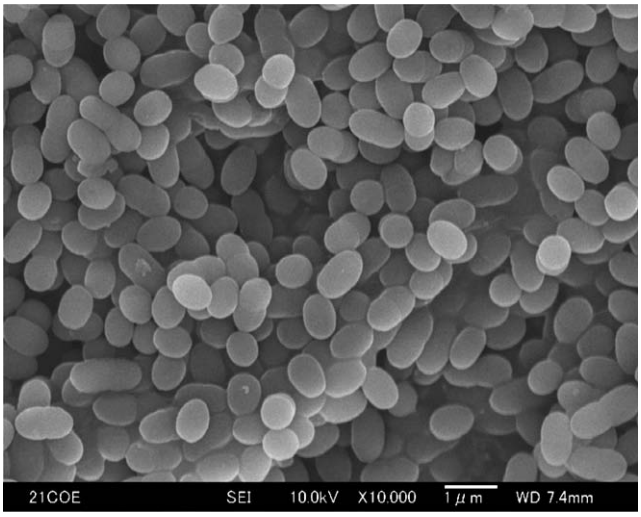

Fig. 1. FE-SEM image of *Lactococcus lactis*.

2.4. Microbial aggregation test

The microbial aggregation of *L. lactis* was determined as follows. Washed or intact cells were re-suspended in a test tube in NaCl aqueous solution in a range of concentrations from 5 to 160 mol/m^3. Cell suspension (5 ml) was added to the sampling tube, and the test tube was agitated at 120 rev/min on the shaker for 20 h. In addition, an aggregation test for washed cells with an excess of added ECP was also carried out. The concentration of ECP was adjusted to give a 10-fold increase compared the ratio measured between ECP and intact cells. The optical density of the aqueous cell suspension was measured at 660 nm using a spectrophotometer (mini photo 10, Sanshin). The percent aggregates of microbial cell, F was calculated using the following equation:

$$F = (1 - OD_t/OD_0) \times 100, \quad (1)$$

where OD_0 is the initial optical density of the cell suspension before agitation, and OD_t is the optical density after agitation. The initial optical density of the cell suspension, A_0 was adjusted to a value in the region of 0.2.