THE ROLE OF EXOPOLYMERS IN BACTERIAL ADHESION

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ABSTRACT

The importance of exopolymers in cell adhesion to a hydrophilic surface was established by studying the adhesion of three mutants of Sphingomonas paucimobilis, high, medium and low polysaccharide producers, to non coated and coated glass with the respective exopolymer. TR and CV were found to produce exopolymers with surfactant properties. These surfactants can easily coat the glass surface, making glass hydrophobic and thus enhancing adhesion. It was hypothesised that the exopolymers bound to the glass surface trough their hydrophilic parts and the exopolymers present at the surface of bacteria can bind together, overcoming the energy barrier created by the negative charge of both surfaces.

KEYWORDS

Adhesion; Sphingomonas paucimobilis; hydrophobicity; free energy of adhesion; exopolymers, biosurfactant

INTRODUCTION

Biofilms can be advantageous in technological processes like in waste water treatment for removal of undesirable components (Lazarova and Manem, 1995). However, they can be undesirable in health due to the occurrence of diseases related to the colonization of bacteria on tissues and implants (Neu et al., 1992; Wilcox, 1993) and in industry on account of the undesirable effect of biocorrosion promoted by microbial cell accumulation at interfaces (Characklis and Cooksey, 1983). Adhesion of microorganisms to solid surfaces is the primary step in the formation of biofilms. Bacteria adhesion is mediated by long range forces, that comprises electrostatic and van der Waals interactions and short range interactions (Oliveira, 1997), such as Born repulsion forces, hydration forces and hydrophobic and steric interactions (Elimelech et al., 1995). Hydrophobicity has been considered the most important short range interaction force in bacterial adhesion (Busscher et al., 1990). The thermodynamic approach of adhesion postulates that when a bacterium and a surface enter into direct contact, the water film present between the interacting surfaces will be removed. Thus the free energy of adhesion ($\Delta G_{adhesion}$) can be calculated by the assumption that the interfaces between bacteria/liquid media (bl) and solid/liquid media (sl) are replaced by a bacteria/solid (b/s) interface (Absolom et al. 1983).

Most of the microorganisms found in aquatic systems possess a net negative charge like most of the adhesion surfaces (Marshall, 1984). Therefore, adhesion can not be mediated only by electrostatic attraction. Thus the explanation for microorganisms to adhere to surfaces in aquatic environments is based on the fact that they can either possess special attaching elements or they can produce extracellular polymers which mediate adhesion. It has been reported in literature that flagella, pili or fimbriae are involved in primary adhesion and

that exopolymers promote irreversible attachment to surfaces (Fletcher and Floodgate, 1972; Marshall, 1984). Many bacteria are able to excrete exopolymers (EPS) forming either tight cell-associated capsules or dispersed slime matrices, which stick out into the aqueous phase (Decho, 1990).

This work presents a study of the initial adhesion to glass of three mutants of *Sphingomonas paucimobilis* that produce high, medium and low amounts of exopolymers. The aim of this study is to correlate the number of the adhered cells with the surface properties of both components involved in the process of adhesion and the amount of exopolysacccharide produced.

MATERIAL AND METHODS

Bacteria preparation

This study was performed with three mutants of *Sphingomonas paucimobilis* (ATCC 31461), a gellan (polysaccharide) producer. The three mutants, TR, CV and F72, were grown in S medium and harvested in the exponential growth phase by centrifugation for 20 minutes at 9000g.

Adhesion medium

The adhesion assays were performed in phosphate saline buffer (PBS 0.1M, pH 7.0) and in the solutions of the excreted and isolated exopolymers of each mutant grown in S medium (EPS). The supernatants were used as the exopolymers solutions, after filtration through a 0.2 μm nitro-cellulose membrane, followed by two days of dialysis against ultra-pure water, using a cellulose membrane with a MWCO of 14 000 Da.

Bacterial exopolymers characterisation

The viscosity of the solution of bacterial exopolymers was measured at room temperature in a capillary viscometer (Cannon Fenske). The surface tension of the solutions of bacterial exopolymers was measured at room temperature in a tensiometer K6 (KRÜSS-Hamburg). The exopolymer content was determined by measuring the dry weight (24h at 80°C) of the precipitate recovered from the solutions of exopolymers after the addition of 2 volumes of chilled ethanol.

Characterisation of bacterial surfaces

The surface tension of the bacterial surface was determined by sessile drop contact angle measurements on bacterial lawns prepared as described by Busscher *et al.* (1984). The presence of a polymeric layer surrounding the surface of the cells of the mutants was observed by epifluorescence microscopy using a lectin (concanavalin A) and calcofluor as labelling agents.

Physico-chemical and physiological characterisation of the adhesion surface

Glass microscope slides were cut into squares of about 1cm^2 and were carefully cleaned. The surface tension was determined by contact angle measurements on bare glass slides and glass slides coated with the exopolymers produced by each mutant.

Adhesion studies

The adhesion assays were performed for each mutant on bare glass slides and on preconditioned glass slides with the respective exopolymer solutions. The adhered cells were enumerated by an automatic image analysis system connected to a microscope (Zeiss-Germany) as described elsewhere (Azeredo *et al.*, 1997a).

RESULTS AND DISCUSSION

Glass is considered an uncoated surface due to its negative charge and to its high hydrophilicity. The exopolymers produced by the mutants TR and CV easily bind to glass because this phenomenon leads to a

decrease in surface free energy. Once coated with the EPS produced by TR and CV, glass becomes hydrophobic (Figure 1). Whereas, glass coated with the EPS produced by F72 remained hydrophilic.
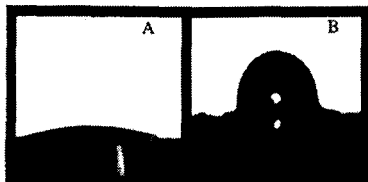

Figure 1. Digitised image of the contact angle of water on glass coated with the solution of expolymer produced by F72 (A) and TR (B).

The values of the free energy of adhesion between cells and bare glass immersed in PBS are positive, meaning that adhesion is thermodynamically unfavourable ($\Delta G_{adhesion} > 0$). Although the thermodynamic model can not predict adhesion between the mutant cells and bare glass, some bacteria did adhere to the glass surface. The number of adhered cells can be directly correlated with cell surface hydrophobicity and the free energy of adhesion (calculated according to van Oss et al., 1987 and Azeredo et al., 1997b respectively) (Figure 2). From Figure 2 it is clear that as the hydrophobicity of the cells increase and the energy of adhesion becomes more negative the higher is the number of adhered cells.

When the adhesion assays were performed using the exopolymer solution as liquid medium, an increase in cell adhesion was obtained: the number of TR adhered cells increased 7 times and CV cells increased 3 times. However, it was not found a great increase on the adhesion of F72 in EPS. The free energy of adhesion and the surface glass hydrophobicity could also be correlated with the number of adhered cells (Figure 2).
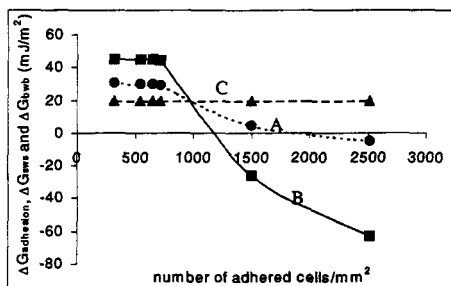

Figure 2. The relationship between the free energy of adhesion of bacteria to glass, $\Delta G_{adhesion}$ (A), glass hydrophobicity, ΔG_{sws}^{tot} (B) and bacteria hydrophobicity, ΔG_{bwb}^{tot} (C) with the number of adhered cells.

The properties of the solutions of exopolymers are described in Table 1. The surface tension of the aqueous solutions of the three types of exopolymers is always lower than that of pure water (72.8 mJ/m2). So, these polymers are acting as surfactants. Biosurfactants have been reported in literature as being antifouling agents when coating hydrophobic surfaces (Velraeds et al., 1996; Neu, 1996). In the present case, the biosurfactant seems to act as a binding agent.

By staining the cell walls with a lectin and calcofluor white it was found that F72 is surrounded by a very thin polymeric layer. The thickness of the polymeric layer is greater for TR followed by CV (Figure 3). The fact that the increase of cell adhesion to coated glass was only observed for bacteria that have a thick polysaccharide layer can be explained by the establishment of polymeric bridges, in which the apolar part of the polymer coating the glass surface would bind to the apolar part of the polymeric cell layer. The polymeric bridges are expected to be necessary to overcome the energy barrier between cells and glass. Close to the surface, adhesion becomes favourable due to the high hydrophobicity of glass and to the negative free energy

of adhesion.

Table 1. Properties of the exopolymer solution produced by each mutant: concentration, viscosity and surface tension (γ_l) measured at room temperature.

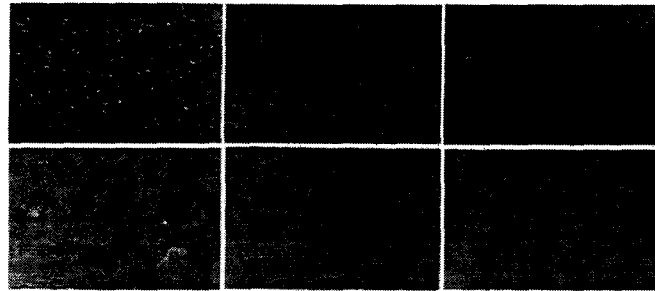| mutant	exopolymer (g/l)	γ_l (mJ/m^2)	Viscosity (cP)
TR	5.31	50.9	1.08
CV	4.33	60.1	1.12
F72	2.50	72.0	0.98

Figure 3 Calcofluor staining of TR (a), CV(b) and F72 (c)and ConA labeling of TR (d), CV(e) and F72(f)

REFERENCES

Absolom, D R, Lamberti, F V, Policova, Z Zing, W, van Oss, C J and Neuman, A W (1983). Surface thermodynamics of bacterial adhesion. *Appl. Environ. Microbiol.*, **46**, 90-97.

Azeredo, J, Meinders, J, Feijó, J and Oliveira, R (1997a). Determination of cell number and size of a population of *Pseudomonas fluorescens* by image analysis. *Biotechnology Techniques*, **11**, 355-358.

Azeredo, J, Ramos, I, Rodrigues, L, Oliveira, R and Teixeira, J (1997b). Yeast flocculation: a new method for characterising cell surface interactions. *J. Institute Brewing.*, **103**, 359-361.

Bussher, H J, Sjollema, J and van der Mei, H (1990). Relative importance of surface free energy as a measure of hydrophobicity in bacterial adhesion to surfaces. In: *Microbial cell surface hydrophobicity*, R J Doyle and M Rosenberg (Eds.), American Society of Microbiology, Washington D.C, pp 335-360.

Busscher, H J, Weerkamp, A H, van der Mei, H, van Pelt, A W, De Jong, HP and Arends, J (1984). Measurement of the surface free energy of bacterial cell surfaces and its relevance for adhesion. *Appl. Environ. Microbiol.*, **48**, 980-983.

Characklis, W G and Coosey, K E (1983). Biofilms and microbial fouling. *Adv. Appl. Microbiol.*, **29**, 93-138.

Decho, A W (1990). Microbial expolymers excretion in ocean environments: their role(s) in food webs and marine processes. *Oceanogr. Mar. Biol. Annu. Rev.*, **28**, 73-153.

Elimelech, M, Gregory, J, Jia, X and Williams, R (1995). *Particle Deposition and Aggregation-Measurements, Modelling and Simulation*. Colloid and Surface Engineering Series, Butterworth-Heinemann Ltd, Oxford.

Fletcher, M and Floodgate, G (1972). An electron-microscopic demonstration of an acidic polysaccharide involved in the adhesion of a Marine bacterium to solid surfaces. *J. Gen. Microbiol.*, **74**, 325-334.

Lazarova, V and Manem, J (1995). Biofilm characterization and activity analysis in water and wastewater treatment. *Wat. Res.*, **29**, 2227-2245.

Marshall, K C (1984). Mechanisms of adhesion. In: *Microbial Cell adhesion and aggregation*, K Marshall (Ed.), Springer-Verlag, Berlin, pp 15-19.

Velraeds, M, van der Mei, H C, Reid, G, Busscher, H (1996). Inhibition of initial adhesion of urogenic Enterococcus faecalis by biosurfactants from Lactobacillus isolates. *Appl. Environ. Microbiol.*, **62**, 1958-1963.

Neu, T R, van der Mei H C and Busscher, H J (1992). Biofilms associated with health. In: *Biofilms-Science and Technology*, L F Melo *et al.* (Eds.), NATO ASI series, Kluwer Academic Publishers, Dordrecht, pp 21-34.

Neu, T R (1996). Significance of bacterial surface active compounds in interaction of bacteria with interfaces. *Microbiol. Rev.*, **60**, 151-166.

Oliveira, R (1997). Understanding adhesion: a mean to prevent fouling. *Experimental Thermal and Fluid Science*, **14**:4, 316-322.

van Oss, C J, Chaudhury, M K and Good,