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The Role of Exopolymers in the Attachment of *Sphingomonas paucimobilis*

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The importance of exopolymers in the adhesion of ***Sphingomonas paucimobilis*** was established by studying the attachment to glass of three mutants with defective gellan production. The attachment assays were performed in either phosphate buffered saline (controls) or in the exopolymeric solutions produced by the mutants. The exopolymer was found to have surface active properties, changing the glass surface from hydrophilic to hydrophobic, making adhesion thermodynamically favourable. Only the cells that had a substantial polymeric layer surrounding their walls were able to significantly colonise glass coated with the exopolymer. It is hypothesised that the exopolymer bound to the glass and the exopolymer present at the surface of the bacteria bound together, overcoming the energy barrier created by the negative charge of both surfaces. It is concluded that the exopolymer from *S. paucimobilis* has a dual role in the process of adhesion by both coating the surface thereby strengthening adhesion and by enhancing adhesion through the establishment of polymeric bridges.

Keywords: attachment; *Sphingomonas paucimobilis*; exopolymers; hydrophobicity; surface active compounds

INTRODUCTION

Adhesion of bacteria to solid surfaces is governed by the physical properties of both the bacteria and the surface and also by the physiological characteristics of bacteria. Surface properties that have been investigated to determine the mechanism of bacterial attachment include surface tension, electric charge and hydrophobicity (Absolom *et al.*, 1983; Busscher *et al.*, 1984; Oliveira, 1997). Hydrophobicity has been considered one of the most important surface properties for adhesion (Busscher *et al.*, 1990; van Loosdrecht *et al.*, 1990; Flemming *et al.*, 1998) because in aqueous media, adhesion is favoured between hydrophobic surfaces, through the elimination of water (van Loosdrecht *et al.*, 1990).

Many bacteria are able to excrete exopolymers (EPS) in the form of either tight cell-associated capsules or dispersed slime matrices (Decho, 1990). The presence of polymeric "footprints" after cell detachment (Neu & Marshall, 1991) and SEM observations showing EPS anchoring the cells to the substratum (Fletcher & Floodgate, 1972) have revealed that they play an adhesive role. The cell attachment process has been described in terms of a reversible adsorption step followed by an irreversible firm attachment (Fletcher & Floodgate, 1972). The formation of this more tenacious anchoring has been associated with the production of EPS. It has also

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been speculated that EPS are involved in initial adhesion by acting as polymeric bridges (van Loosdrecht *et al.*, 1990).

Sphingomonas paucimobilis was chosen for the present study because it is a ubiquitous bacterium in soil, water and sediments. This bacterium has great potential in soil bioremediation and waste water treatment due to its capacity to degrade aromatic compounds (Fredrickson *et al.*, 1995). The strain ATCC 31461 (Yabuuchi *et al.*, 1990) is able to produce large amounts of an anionic EPS called gellan. This substance is composed of repeated units of a tetrasaccharide with two molecules of D-glucose, one of D-glucoronic acid and one of L-rhamnose (Kang & Veeder, 1982; Pollock, 1993; Chandrasekaren & Radha, 1995). Moreover, well-defined mutants defective in EPS production (Richau *et al.*, 1997a; 1997b) are available that constitute powerful tools to investigate the importance of EPS in bacterial attachment.

This paper reports a study on the initial attachment to glass of three mutants of *S. paucimobilis* producing high, medium and low amounts of EPS. The objective of the study was to determine the effect of the EPS and the surface properties of both mutants and glass on the process of attachment.

The mutants used were isolated by Richau *et al.* (1997a) after prolonged Cu^{2+} stress growth conditions. The mutants are defective in gellan production as a result of decreased activity of enzymes involved in the synthesis of repeated tetrasaccharide units of gellan and consequently in its polymerisation (Richau *et al.*, 1997b). It should be noted that the mutants used in this study produce the same EPS, but in different amounts and with different degrees of polymerisation (Richau *et al.*, 1997a; 1997b).

MATERIALS AND METHODS

Bacterial Strains

The gellan producing strain *S. paucimobilis* (ATCC 31461) was used to derive three mutant cells (TR, CV and F72) deficient in gellan synthesis after Cu^{2+}-stressed cultivation as described by Richau *et al.* (1997a). The mutant cells were supplied by Instituto Superior Técnico, Portugal.

Bacterial Cultivation and Preparation

The three mutants, TR, CV and F72, were grown in S medium designed to stimulate polysaccharide production and in a standard LB medium. S medium contained l^{-1} of distilled water, 12.5 g $Na_2HPO_4 \cdot 2H_2O$, 3 g KH_2PO_4, 1 g NaCl, 1 g K_2SO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2 \cdot 2H_2O$, 0.001 $FeSO_4 \cdot 7H_2O$, 5g glucose, 1g yeast extract (DIFCO) and 1 g casein (DIFCO); LB medium contained 10 g peptone (DIFCO), 5 g yeast extract (DIFCO) and 5 g NaCl l^{-1} of distilled water. The cells were harvested in the exponential growth phase by centrifugation for 20 min at 9000 g and washed three times with ultra-pure water and two times with chilled 0.1 M phosphate buffered saline (PBS) pH 7.0 (0.29 g l^{-1} KH_2PO_4, 1.19 g l^{-1} K_2HPO_4, 4.93 g l^{-1} NaCl). After that, they were preserved in filtered (0.2 μm pore size) PBS (0.1 M, pH 7.0) at −70°C.

Attachment Medium

The attachment assays were performed in two different media, *viz.* PBS (0.1 M, pH 7.0) and in solutions of the excreted and isolated EPS of each mutant grown in S medium. The supernatants of the above centrifugation procedure were used as the EPS solutions, after filtration through a 0.2 μm nitro-cellulose membrane, followed by dialysis for 2 d against ultra-pure water, using a cellulose membrane with a MWCO of 14 kDa.

Bacterial EPS Characterisation

The surface tension of the solutions of bacterial EPS was determined with a ring tensiometer (K6, KRÜSS-Hamburg) at room temperature and is given as a mean value of 10 measurements. The EPS content was determined by measuring

the dry weight (24 h at 80°C) of the precipitate recovered from the solutions after addition of 2 volumes of chilled ethanol.

Physico-chemical 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 measurements were carried out at room temperature using water, di-iodomethane and glycerol as reference liquids. Determination of contact angles was performed automatically with the aid of an image analysis system (Kruss-GmbH, Hamburg). The images were received by a video camera connected to a 486 DX4 100 MHz personal computer, with an automatic measuring system (G2/G40). At least 20 measurements were done for each liquid and each surface. The zeta potential of the mutants was measured in PBS and in the exopolymer solutions by means of a Zeta-Meter 3.0+.

The presence of a polymeric layer surrounding the surface of the cells of each mutant was observed by epifluorescence microscopy using a lectin and calcofluor as labelling agents. The lectin used was fluorescein isothiocyanate concanavalin A (conA-FITC) which is specific for terminal α-D-mannose and α-D-glucose. When coupled to the fluorescent molecule FITC, the complex formed exhibits a green colour after excitation at 490 nm. Calcofluor white is a fluorescent dye specific for polysaccharides containing (1-3)-β and (1-4)-β-D-glucopyranolyl groups. This dye exhibits a blue colour when excited at 440 nm. Aliquots of mid-log-phase cultures of CV, TR and F72 grown in S and LB medium were centrifuged at 9000 g for 20 min. The pellets were washed with chilled PBS (0.1 M pH 7.0) and resuspended in PBS to a final concentration given by an absorbance of 0.3 measured at 640 nm. A volume of 50 µl of a solution of 1 g l^{-1} of ConA-FITC was added to 1 ml of each suspension. The ConA-FITC solution was prepared with filtered (0.2 µm) ultra-pure water. After 30 min incubation at room temperature, the cells were washed three times with PBS by centrifugation at 14000 rpm for 5 min and resuspended in 1 ml of PBS. For calcofluor white staining the same procedure was followed except that the cell suspensions were incubated with 10 µl of a solution of the dye (50 mM prepared with ultra-pure water filtered through a 0.2 µm pore membrane).

Attachment Surface

Glass microscope slides were cut into squares of about 1 cm^2 and carefully cleaned by immersion in concentrated chromosulfuric acid for 24 h, after which the slides were well rinsed with distilled water. The slide surface was then degreased with a detergent followed by alternate rinsing with methanol and demineralised water.

Physico-chemical Characterisation of Glass Surfaces

The surface tension was determined by contact angle measurements with water, glycerol and di-idomethane on clean glass slides and glass slides coated with the EPS produced by each mutant. The coating was performed by immersing the glass slides in each EPS solution for 30 min. The slides were carefully withdrawn from the solutions and allowed to dry over 48 h at 30°C in a Petri dish. Electrophoretic mobility was measured using fine particles of crushed glass immersed in PBS and in each solution of bacterial EPS (TR, CV and F72) using a Zeta-Meter 3.0+. At least 40 measurements were made for each sample.

Attachment Assays

The attachment assays were performed for each mutant on clean glass slides and on preconditioned glass slides with the respective EPS solution. For each mutant the glass slides were immersed for 30 min in PBS (0.1 M, pH 7.0)

and in the corresponding solution of EPS. Four bacterial cell suspensions of each mutant (0.5×10^8 cells ml^{-1}) were prepared by resuspending pellets of cells grown in S and LB medium in a solution of PBS and in the EPS solution. The preconditioned glass slides were incubated at 30°C in each type of cell suspension under gentle agitation (100 rpm). After 2 h incubation, the slides were withdrawn from the suspensions and rinsed gently for 30 s in 0.1 M PBS (filtered through a 0.2 μm membrane) to remove non-attached cells. The rising was performed by moving the disks slowly through the washing solution. The washing procedure was performed carefully to avoid the transfer of cells from the glass surface to the water/air interface. The rinsed slides were dried in Petri dishes at 30°C for 24 h. The adhering cells were enumerated by an automatic image analysis system connected to a microscope (Zeiss-Germany) as described elsewhere (Azeredo *et al.*, 1997). For each experiment, eight plates were analysed and 10 images of each plate were taken, giving a total number of 80 images analysed. The attachment assays were repeated 4 times.

RESULTS

Attachment Assays

The attachment assays revealed that the medium greatly influenced the attachment capability of the mutants (Table I). In PBS the mutant F72 was able to colonise glass to a greater extent than mutants TR and CV. However, in the presence of the EPS solution from F72 grown in S medium, it colonised glass to a lesser extent than either of the other two mutants, also grown in S medium. Mutant TR (grown in S medium) was able to colonise glass coated with EPS to a seven times greater extent than in the control PBS and the mutant CV (grown in S medium) to a three times greater extent. The medium in which the mutants were grown also influenced their abilities to colonise glass (Table I); TR and CV grown in S medium showed a greater ability to colonise glass than when grown in LB medium.

TABLE I Number of bacteria mm^{-2} (in LB and S medium) adhered to glass in PBS and in the respective solutions of EPS (± SD)

	Adhesion medium			
	PBS		EPS	
	Growth medium		Growth medium	
Mutants	LB	S	LB	S
TR	251 ± 36	323 ± 36	718 ± 72	2513 ± 215
CV	323 ± 72	539 ± 72	718 ± 71	1508 ± 144
F72	431 ± 72	646 ± 72	718 ± 70	826 ± 72

In order to explain the results obtained, the surface properties of the mutants and the substratum were studied in relation to the number of attached cells.

Physico-chemical Properties of the Interacting Surfaces

The three mutants are negatively charged (negative values of zeta potential). However when immersed in the respective EPS solution the zeta potential values became less negative compared to those recorded in PBS (Table II). The same behaviour was observed for glass particles in contact with the EPS solutions (Table II).

The total surface tension values (γ^{tot}) and the relative contributions of Lifshits-van der Waals (LW) and the electron donor (γ^-) and electron acceptor (γ^+) parameters of the Lewis-acid base (AB) interactions were calculated using the approach of van Oss *et al.* (1987). From the data shown in Table III, it is apparent that the polar component (γ^{AB}) of the surface tension of the mutants had a greater contribution to the overall surface tension than the apolar component (γ^{LW}). The values for the surface tension of glass are in good agreement with those reported by van Oss and Giese (1995), showing the great polarity of this surface. However, when coated

TABLE II Zeta potential values (ζ) of the mutants TR, CV and F72 and the zeta potential of glass immersed in PBS and in the respective solutions of EPS (± SD)

	ζ (mV)			
	Surfaces			
Liquid media	TR	CV	F72	Glass
PBS	−88.5 ± 12	−93.2 ± 12	−88.3 ± 13	−32.1 ± 10
EPS_{TR}	−38.2 ± 5			−13.9 ± 2
EPS_{CV}		−40.7 ± 5		−14.1 ± 2
EPS_{F72}			−20.7 ± 5	−7.5 ± 1.9

TABLE III The apolar component (γ^{LW}) and electron donor (γ^-) and electron acceptor (γ^+) parameters of the polar component (γ^{AB}) of the surface tension (γ^{tot}) of the mutants TR, CV and F72 and of clean glass and glass coated with EPS (± SD)

	γ^{LW} (mJ m^{-2})	γ^+ (mJ m^{-2})	γ^- (mJ m^{-2})	γ^{AB} (mJ m^{-2})	γ^{tot} (mJ m^{-2})
Mutants					
TR	22.9 ± 1.1	5.8 ± 0.7	49.0 ± 1.8	33.7 ± 2.1	56.6 ± 3.2
CV	24.6 ± 1.6	6.2 ± 0.8	49.0 ± 2.2	34.9 ± 2.4	59.5 ± 4.0
F72	27.4 ± 1.1	6.0 ± 1.1	49.0 ± 1.6	34.3 ± 3.2	61.7 ± 4.3
Glass					
Clean	33.0 ± 1.2	1.0 ± 0.2	63.5 ± 0.2	15.9 ± 1.6	48.9 ± 2.8
Coated with EPS_{TR}	19.0 ± 1.7	1.3 ± 0.5	1.1 ± 0.4	2.4 ± 0.6	21.4 ± 2.3
Coated with EPS_{CV}	20.7 ± 1.1	2.4 ± 0.3	10.2 ± 2.7	9.9 ± 1.4	30.6 ± 2.5
Coated with EPS_{F72}	32.7 ± 1.1	1.0 ± 0.3	62.5 ± 1.1	15.8 ± 2.3	48.5 ± 3.4

with the EPS solutions produced by TR and CV, the polar component of the surface tension showed a significant decrease on account of the large decline in the electron donor component. This fact indicates that the EPS from the greatest gellan producers (TR and CV) decreased the polarity of the glass surface.

The surface hydrophobicity of the mutants and glass was calculated with the surface tension values from Table III, using the method proposed by van Oss and Giese (1995). According to these authors, the hydrophobicity of a solid can be evaluated as the free energy of interaction between the molecules of two of its surfaces (s) when immersed in water (w), represented as ΔG^{tot}_{SWS}. This expresses the degree to which the attraction of the solid molecules to water is greater (hydrophilicity) or smaller (hydrophobicity) than the attraction that water molecules have to each other. Thus, when the global free energy of interaction between the molecules of the solid surfaces immersed in water is repulsive ($\Delta G^{tot}_{SWS} > 0$) the solid is considered hydrophilic. On the contrary, the more negative ΔG^{tot}_{SWS} is, the higher is the solid hydrophobicity. Considering this notation, the three mutants and clean glass were hydrophilic (Table IV). However, when coated with the EPS from mutant CV, glass became hydrophobic and strongly hydrophobic when coated with the EPS produced by TR.

The Influence of Surface Properties on Attachment

Direct contact between a bacterium and a surface in aqueous medium is achieved when the water film in between is removed. This is described by the Dupré equation, from which the Gibbs free energy of adhesion ($\Delta G_{adhesion}$) can be calculated, assuming that the interfaces bacteria(b)/liquid(1) medium and solid(s)/

TABLE IV The hydrophobicity of the mutants and of glass (ΔG^{tot}_{SWS}) and of the apolar (ΔG^{LW}_{SWS}) and polar (ΔG^{AB}_{SWS}) components (± SD)

	ΔG^{LW}_{SWS} (mJ m^{-2})	ΔG^{AB}_{SWS} (mJ m^{-2})	ΔG^{tot}_{SWS} (mJ m^{-2})
Mutants			
TR	−0.03 ± 0.05	+20.6 ± 3.5	+20.6 ± 3.6
CV	−0.2 ± 0.2	+20.0 ± 3.9	+19.8 ± 4.1
F72	−0.6 ± 0.2	+20.3 ± 5.3	+19.6 ± 5.5
Glass			
Clean	−2.3 ± 0.4	+47.3 ± 2.7	+45.0 ± 3.1
Coated with EPS_{TR}	−0.2 ± 0.2	−62.6 ± 0.1	−62.8 ± 0.3
Coated with EPS_{CV}	−0.03 ± 0.06	−26.0 ± 1.1	−26.0 ± 1.2
Coated with EPS_{F72}	−2.2 ± 0.4	+46.2 ± 4.1	+44.0 ± 4.5

liquid(l) medium are replaced by a bacteria(b)/solid(s) interface (Absolom *et al.*, 1983). The thermodynamic theory postulates that adhesion is favourable when $\Delta G_{adhesion} < 0$ (Absolom *et al.*, 1983).

The values of the free energy of adhesion between each type of cell and bare glass immersed in PBS were positive (Table V), indicating that adhesion is thermodynamically unfavourable ($\Delta G^{tot}_{bls} > 0$). These positive values are mainly due to the repulsion conferred by the AB parameter (ΔG^{AB}_{bls}) vis-à-vis a LW attraction (ΔG^{LW}_{bls}) that is almost negligible. According to the thermodynamic model, adhesion of TR (high gellan producer) to coated glass with its EPS is favourable because the ΔG^{tot}_{bls} value is negative. The free energy of interaction of CV (medium producer) with coated glass is less positive than the free energy of interaction between F72 and coated glass.

TABLE V The free energy of adhesion between the mutants and glass (clean and coated) ($\Delta G_{adhesion}$) and of the respective apolar (ΔG^{LW}_{bls}) and polar (ΔG^{AB}_{bls}) components (± SD)

Interacting bodies (glass/bacteria)	ΔG^{LW}_{bls} (mJ m^{-2})	ΔG^{AB}_{bls} (mJ m^{-2})	$\Delta G_{adhesion}$ (mJ m^{-2})
Clean/TR	−0.3 ± 0.2	+31.2 ± 1.3	+30.9 ± 1.5
Clean/CV	−0.6 ± 0.4	+30.8 ± 1.5	+30.2 ± 1.9
Clean/F72	−1.2 ± 0.3	+31.0 ± 1.2	+29.8 ± 1.5
$Coated_{EPSTR}$/TR	+0.03 ± 0.1	−4.7 ± 2.4	−4.7 ± 2.5
$Coated_{EPSCV}$/CV	+0.05 ± 0.1	+4.9 ± 2.4	+5.0 ± 2.5
$Coated_{EPSF72}$/F72	−1.0 ± 0.2	+30.5 ± 1.4	+29.5 ± 1.6

The Influence of EPS on Bacterial Attachment

The solutions recovered after growing the mutants in S medium had different concentrations of exopolymer (Table VI). This was an expected result because the mutants are defective to different degrees in gellan synthesis (Richau *et al.*, 1997b). The surface tensions of the EPS solutions had values inferior to that of water (72.8 mJ m^{-2}), which means that the exopolymers have surface active properties. TR, the highest gellan producer (Richau *et al.*, 1997a), was able to generate a solution with the greatest concentration of EPS, and thus displayed the lowest surface tension, followed by the mutant CV. F72 excreted smaller amounts of EPS giving rise to solutions with surface tensions closer to that of water (Table VI).

The presence of a polymeric layer surrounding the mutants was inspected by epifluorescence microscopy. The observations revealed that TR and CV cells grown in S medium exhibited a high fluorescence around their cell walls. F72 grown in S medium and all the mutants grown in LB medium showed little fluorescence intensity. From these results it is apparent that the surfaces of all the mutants grown in LB medium as well as F72 grown in S medium were surrounded by a very thin polymeric layer. On the contrary, when grown in S medium, the thickness of the polymeric layer greatly increased in TR, followed by CV.

TABLE VI The concentration and surface tension (γ_l) of the exopolymer solutions produced by each mutant measured at room temperature

Mutant	Exopolymer(g l^{-1})	γ_l(mJ m^{-2})
TR	5.31	50.9
CV	4.33	60.1
F72	2.50	72.0

DISCUSSION

The differences in the properties of the EPS solutions obtained after TR, CV and F72 growth in S medium (Table VI) are mainly due to the amount of the exopolymer produced by the mutants. The surface tension of the EPS solutions from TR and CV revealed that the exopolymer produced by these mutants has surface-active properties. The fact that the value of the surface tension of the EPS_{F72} solution was similar to that of water probably means that the amount of exopolymer is insufficient to be able to promote a decrease in the surface tension.

Surface-active compounds (SACs) are molecules formed by hydrophilic and hydrophobic components, which tend to interact with interfaces. Polysaccharides containing 6-deoxy sugars like rhamnose, as in the case of gellan, are considered polyphilic polymers with hydrophobic groups distributed across the entire molecule (Neu, 1996). Synthetic SACs have been used to reduce the adherence of cells to hydrophobic surfaces (Paul & Jeffrey, 1985; Rosenberg *et al.*, 1989; Stelmack *et al.*, 1999). Microbial SACs have been reported to play an important role in bacterial attachment as they can either inhibit attachment to hydrophobic surfaces (Velraeds *et al.*, 1996), or enhance attachment to hydrophilic surfaces (Neu, 1996). So, it would be expected that the EPS produced by *S. paucimobilis* influences its attachment to surfaces. The greatest EPS producer (mutant TR) was able to colonise glass to a greater extent than the lowest EPS producer (mutant F72) (Table I). Moreover, this greater ability to colonise glass was only seen in the presence of EPS.

The EPS produced by these mutants promoted changes in the surface properties of the interacting surfaces (cells and glass). The decrease in the zeta potential of glass when immersed in the EPS solution (Table II) can be explained by the displacement of the shear plane outwards. This may be caused by the presence of polymers at the surface (Elimelech *et al.*, 1995), assuming that the EPS in solution would spontaneously coat the glass particles. It is well documented that SACs are able to spontaneously coat surfaces (Neu, 1996).

The EPS produced by the mutants also promoted changes in the hydrophobicity of the glass surface. As the EPS solution of mutant F72 had a lower content of exopolymer, no significant alteration was detected on glass hydrophobicity. Recently, the establishment of a direct relationship between the degree of surface hydrophobicity and its subsequent colonisation has shown the importance of hydrophobicity in bacterial attachment (Teixeira & Oliveira, 1998; 1999).

From the thermodynamic point of view, adhesion is only favourable for mutant TR and glass coated with its EPS (Table V), and this was the assay where the greatest degree of colonisation was observed. However, in all experiments colonisation also occurred though to a lesser extent. According to other authors, this criterion cannot be used straightforwardly (Busscher & Weerkamp, 1987), since in many cases attachment occurs when $\Delta G_{adhesion} > 0$. Nevertheless, in such situations, a decrease in $\Delta G_{adhesion}$ is followed by an increase in attachment (Busscher & Weerkamp, 1987; Sousa *et al.*, 1997; Teixeira *et al.*, 1997; 1999). The same tendency was observed in the present study (Tables I and V).

The medium in which the mutants were grown significantly influenced their ability to colonise glass. In S medium (designed to enhance EPS production), a thick polysaccharide layer was formed around the cells of the mutants producing more EPS. In LB medium this layer was not observable. Taking into account the attachment results (Table I), an increase in the degree of surface colonisation is associated with the presence of a polymeric slime layer surrounding the cell wall. The colonisation of glass by the mutants grown in LB medium did not occur to a large extent compared with the mutants grown in S medium. So, the presence of a polymeric layer was a

determinant for attachment. It can be hypothesised that attachment between the mutants producing more EPS and coated glass occurred through the establishment of polymeric bridges between the molecules of the exopolymer anchored to the glass surface and the exopolymer molecules present at the cell wall surface. van Loosdrecht *et al.* (1990) described the importance of polymeric bridges in overcoming the energy barrier created by the repulsive electrostatic interactions. As both mutants and glass are highly negatively charged an energy barrier is expected to be present, thereby avoiding close contact.

CONCLUSIONS

The EPS produced by *S. paucimobilis* possesses surface-active properties. SACs are characterised by having polar and apolar portions that interact with interfaces. So the molecules present in the aqueous EPS solutions can easily bind to a glass surface. Once at the glass surface, the EPS molecules anchor throughout their polar portion and convert the hydrophilic glass into a hydrophobic surface that favours attachment. Only bacterial cells that have a substantial polymeric layer surrounding their walls were able to adhere in significant numbers to glass coated with EPS and thus hydrophobic surfaces. So, it can be expected that attachment is enhanced by the establishment of polymeric bridges in which the apolar portions of the polymer coating the glass surface would bind to the apolar portions of the polymeric capsular layer. The polymeric bridges are thought to be necessary to overcome the energy barrier created by the electrostatic repulsion between glass and cells. Close to the glass surface, attachment becomes favourable due to the high hydrophobicity of glass, leading to a thermodynamically favourable process.

The exopolymer from *S. paucimobilis* has a dual role in attachment; it can coat the surface and thus strengthens attachment and it also enhances attachment by the establishment of polymeric bridges.

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References

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

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

Busscher H J, Weerkamp A H (1987) Specific and non-specific interactions in bacterial adhesion to solid substrata. *FEMS Microbiol Rev* **46**: 167–173

Busscher H J, Sjollema J, van der Mei H C (1990) Relative importance of surface free energy as a measure of hydrophobicity in bacterial adhesion to surfaces. In: Doyle R J, Rosenberg M (eds) *Microbial Cell Surface Hydrophobicity*. American Society of Microbiology, Washington DC, pp 335–360

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

Chandrasekaran R, Radha A (1995) Molecular architectures and functional properties of gellan gum and related polysaccharides. *Trends Food Sci Technol* **6**: 143–148

Decho A W (1990) Microbial exopolymers 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, Williams R (1995) Particle deposition and aggregation-measurements, modelling and simulation. *Colloid and Surface Engineering Series*, Butterworth-Heinemann Ltd, Oxford

Fredrickson J K, Balkwill D L, Drake G R, Romine M F, Ringelberg D B, White D C (1995) Aromatic-degrading *Sphingomonas* isolates from deep subsurface. *Appl Environ Microbiol* **61**: 1917–1922

Flemming C A, Palmer R J Jr., Arrange A A, van der Mei H C, White D C (1998) Cell surface physicochemistry alters biofilm development of *Pseudomonas aeruginosa* lipopolysaccharide mutants. *Biofouling* **13**: 213–231

Fletcher M, 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

Kang K S, Veeder G T (1982) Gellan polysaccharide S-60 and bacterial fermentation process for its preparation. US patent 4,326,053

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

Neu T R, Marshall K C (1991) Microbial "footrprints" – a new approach to adhesive polymers. *Biofouling* **3**: 101–112

Oliveira R (1997) Understanding adhesion: a means to prevent fouling. *Exp Thermal Fluid Sci* **14**: 316–322

Paul J H, Jeffrey W H (1985) The effect of surfactants on the attachment of estuarine and marine bacteria to surfaces. *Can J Microbiol* **31**: 224–228

Pollock J T (1993) Gellan-related polysaccharides and the genus *Sphingomonas*. *J Gen Microbiol* **193**: 1939–1945

Richau A J, Choquenet D, Fialho A M, Sá-Correira I (1997a) Emergence of Cu^{++}-tolerant mutants defective in gellan synthesis in Cu^{++}-stressed cultures *of Sphingomonas paucimobilis*. *Res Microbiol* **148**: 251–261

Richau J A, Choquenet D, Fialho A M, Moreira L M, Sá-Correia I (1997b) The biosynthesis of the exopolysaccharide gellan results in the decrease *of Sphingomonas paucimobilis* tolerance to copper. *Enzyme Microb Technol* **20**: 510–515

Rosenberg M, Tal M, Weiss E, Guendelman S (1989) Adhesion of noncoccal dental plaque microorganisms to buccal epithelial cells inhibition by saliva and amphipathic agents. *Microb Ecol Health Dis* **2**: 197–202

Sousa M, Azeredo J, Feijó J, Oliveira R (1997) Polymeric supports for the adhesion of a consortium of autotrophic nitrifying bacteria. *Biotech Technol* **11**: 755–758

Stelmack P L, Gray M R, Pickard M A (1999) Bacterial adhesion to soil contaminants in the presence of surfactants. *Appl Environ Microbiol* **65**: 163–168

Teixeira P, Oliveira R (1998) The importance of surface properties in the selection of supports for nitrification in air-lift bioreactors. *Biophys Eng* **19**: 143–147

Teixeira P, Oliveira R (1999) Influence of surface characteristics on the adhesion of *Alcaligenes denitrificans* to polymeric substrates. *J Adhesion Sci Technol* **13**: 1287–1294

van Loosdrecht M, Norde W, Lyklema L, Zehnder J (1990) Hydrophobic and electrostatic parameters in bacterial adhesion. *Aquat Sci* **51**: 103–114

van Oss C J, Giese R F (1995) The hydrophilicity and hydrophobicity of clay minerals. *Clay Minerals* **43**: 474–477

van Oss C J, Chaudhury M K, Good R J (1987) Monopolar Surfaces. *Adv Coll Int Sci* **28**: 35–64

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

Yabuuchi E, Yano I, Oyaizu H, Hashimoto Y, Ezaki T, Yamamoto H (1990) Proposals of *Sphingomonas paucimobilis* gen nov and comb nov, *Sphingomonas parapaucimobilis* sp nov, *Sphingomonas yanoikuyae* sp nov, *Sphingomonas adhaesiva* sp nov, *Sphingomonas capsulata* comb nov, and two genospecies of the genus *Sphingomonas*. *Microbiol Immunol* **34**: 99–119