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BACTERIAL TRANSPORT AND COLONIZATION IN LOW NUTRIENT ENVIRONMENTS

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Abstract—Crucial and potentially rate limiting events in biofilm formation are the transport of microorganisms to the solid–water interface and the subsequent attachment onto a substratum. If these attached cells find suitable environmental conditions they will replicate, grow and form a biofilm. Experiments with different surfaces—stainless steel, glass, and polycarbonate—and two *Pseudomonas* species were conducted and parameters describing attachment, growth, and transport were measured *in situ* and in real time using image analysis techniques. The phenomenon of cellular starvation was investigated with special attention to the effect on the attachment characteristics of the two tested *Pseudomonas* species. Experiments were conducted with *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* as a Mot+ and a Mot− strain. As a result of superior transport properties, flagellated cells of *Pseudomonas fluorescens* colonized a substratum at a higher rate than unflagellated cells. Nutrient conditions in the bulk water influenced the growth of suspended and attached organisms, as well as the cellular transport to the solid–water interface and the rate of attachment onto the substratum. Starved cells of *P. aeruginosa* and *P. fluorescens* exhibited increased cell motility by size reduction. Cellular transport was demonstrated to be a crucial process in microbial colonization, and may be limiting the overall rate of surface colonization. The phenomenon was demonstrated for a motile and nonmotile mutant of the same strain. Hence, diffusive, advective, and hydrodynamic properties of a specific system cannot be neglected if we try to understand and simulate microbial colonization. Copyright © 1996 Elsevier Science Ltd

Key words—biofilm formation, initial cell surface colonization, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, stainless steel, cell transport, motility, starvation, image analysis, *in situ*, real time

INTRODUCTION

Oligotrophic conditions inherent in drinking water, industrial pure water, and certain cooling water distribution systems preclude extensive planktonic microbial growth (Marshall, 1985). Organic carbon levels are low and residual disinfectants may further reduce the potential for suspended cell survival (McFeters, 1989; LeChevallier and McFeters, 1990). If nutrient concentrations are sufficiently low, starvation of suspended cells may occur (Morita, 1982; 1988). However, these environments may favor the presence of bacterial biofilms. Microbial growth in biofilms is enhanced over suspended growth due to the flux of substrates to the cells (Kjelleberg *et al.*, 1982; Kjelleberg and Hermansson, 1984). Nutrient availability on surfaces may be higher due to surface-associated organic material (LeChevallier and McFeters, 1990; Davies and McFeters, 1988). Cells need not be firmly attached to a substratum to benefit from the nutrient-enriched state at the solid–liquid interface (Marshall, 1979). Biofilm systems can also enhance cell survival with respect to biocides or other chemical stressors (LeChevallier and McFeters, 1990; Van der Wende *et al.*, 1990; Chen *et al.*, 1992). An attached form of growth is beneficial to the organisms, especially under low nutrient conditions.

The processes occurring during microbial colonization and biofilm formation on a solid–liquid interface are summarized by Mueller *et al.* (1992) as: substratum conditioning by organic molecules; transport of cells to the surface; adsorption of cells to the substratum; transformation of reversibly adsorbed cells to irreversibly adsorbed cells; growth; and erosion of cells. Process analysis techniques have been used to mathematically model the processes that occur during colonization (Powell and Slater, 1983; Escher and Characklis, 1990; Mueller *et al.*, 1992). Variables such as mass, energy, and momentum have been combined with stoichiometry, reaction rate, and transport coefficients to form predictive models.

The coefficients responsible for attachment of cells vary with environmental parameters such as temperature, pH, concentration of trace elements, the

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composition and balance of essential nutrients and their ratio to carbon, the ionic strength of the medium, and the presence or absence of specific ions such as calcium and magnesium (Abbott *et al.*, 1983; Paul and Jeffrey, 1985; Pedersen *et al.*, 1986; Turakhia, 1986; Stanley, 1983). Abiotic variables have been used to describe the physical and chemical environment in which the microbial colonization is occurring. Phenomena governing the process of bacterial colonization have been shown to be influenced by microbial and surface characteristics. For example, adsorption can be influenced by compounds such as chlorine and chloramine (Hermanowicz and Filho, 1992). Surface active chemicals also influence microbial attachment (Whitekettle, 1991). Cellular adsorption is mediated by cell surface hydrophobicity, bacterium–substratum charge interactions, surface roughness, and surface free energy (Vanhaeke *et al.*, 1990; Van Loosdrecht *et al.*, 1990; Mueller *et al.*, 1992). Firm attachment following initial adhesion, although not clearly understood, may include specific cell surface structures (e.g. fibrils or polymers) (Geesey, 1982).

All of these interactions may be limited by the transport of bacteria to a surface, which is primarily a function of cellular diffusivity, hydrodynamics, and the system geometry. Cellular diffusivity is governed by bacterial properties such as size and motility; a small, motile bacterium will be characterized by a higher diffusivity value than a large, nonmotile cell. Transport could potentially be the rate limiting step in colonization, and an understanding of the importance of the bacterial growth state may provide insight into predicting and controlling microbial fouling.

THEORY

Cellular diffusivity is considered to be the primary transport process for bacteria through the laminar boundary layer at the solid–liquid interface and values have been calculated by Jang and Yen (1985). Using the cellular properties of motility and the length of the random free run for the organism the following equation was derived:

$$D_c = \frac{v_r \cdot d_r}{3 \cdot (1 - \cos \gamma)}, \tag{1}$$

where D_c = diffusivity [$L^2\,t^{-1}$], v_r = velocity of motility [$L\,t^{-1}$], d_r = length of random free run [L], and γ = main angle of turn; assuming no chemotaxis: $\cos \gamma = 0$.

This equation allows for the calculation of nonBrownian diffusivity from cellular motility data for a specific species. For example, the nonBrownian diffusivity for *Pseudomonas aeruginosa* based on data from Vaituzi and Doetsch (1986) ($v_r = 55.8\ \mu m\ s^{-1}$, $d_r = 500$–$85\ \mu m$) is $10^{-3}\ mm^{-2}\ s^{-1}$. In comparison, the diffusivity for nonmotile *P. aeruginosa* as calculated by the Stokes–Einstein equation (Brownian diffusion alone) at 22°C is $4 \times 10^{-7}\ mm^2\ s^{-1}$. Thus, transport of motile *P. aeruginosa* cells is superior to that of their nonmotile counterparts.

If a cell is transported to the surface in a system with fluid flow, it must then form a cell–surface association to remain on the substratum. Sticking efficiency, defined as the ratio of cells adsorbing to the total number of cells colliding with the surface, was used by Escher and Characklis (1990) to quantify the adsorption potential of a particular microbial species to a substratum. A mathematical description of sticking efficiency was developed from a model of Bowen *et al.* (1976), who proposed an analysis to describe particle deposition from a suspension under fully developed laminar flow in a parallel plate channel. Sticking efficiency is determined by separating the transport and sorption processes when the cell diffusivity, system geometry and hydrodynamics are known. Values vary from 0 (no cells adsorb) to 1 (all cells transported to the surface adsorb). Since sticking efficiency is independent of transport and depends only on cell–surface interactions, it can be used to compare bacterial adsorption at various interfaces or in environments independent of cellular transport phenomena. Comparisons may also be made between different bacterial species or the same organism at varying stages in the growth cycle.

Cellular adsorption rate is primarily the result of two processes; cellular transport rate and sticking efficiency. A specific rate coefficient for adsorption can be calculated by normalizing the rate of adsorption onto the substratum for the cell concentration in the bulk water (Mueller *et al.*, 1992). Adsorption and desorption rates are driven by the bulk water cell concentration, while growth and erosion rates are dependent on the irreversibly attached cell surface concentration:

$$\Phi = \frac{r_a}{N_s} \tag{2}$$

$$K_a = \frac{r_a}{X_b'} \tag{3}$$

$$K_d = (1 - \alpha) K_a \tag{4}$$

$$\mu_s = \frac{r_g}{X''} \tag{5}$$

$$K_e = \frac{r_e}{X''} \tag{6}$$

where: Φ = sticking efficient [–], N_s = total flux of cells to the substratum [cell no. $L^{-2}\,t^{-1}$], K_a, K_d = specific rate coefficient of adsorption and desorption, respectively [$L\,t^{-1}$], μ_s, K_e = specific rate of growth and erosion on the substratum, respectively [t^{-1}], r_a, r_d, r_g, r_e = rate of cell adsorption, desorption, growth, and erosion, respectively [cell no. $L^{-2}\,t^{-1}$], X_b' = bulk water cell concentration [cell no. L^{-3}], X'' = cell surface concentration [cell no. L^{-2}], and $(1 - \alpha)$ = probability of desorption [–].

K_a is independent of bulk water cell concentration but dependent on geometry and hydrodynamics of the flow system, as well as the diffusivity and inherent

adsorptive capabilities of the cells. The adsorption rate coefficient is a useful parameter for comparing the propensity of an organism to adsorb under specific environmental conditions. The adsorption rate coefficient was shown to be independent of bulk water cell concentration for cell densities below 10^7 CFU ml^{-1} (Escher and Characklis, 1990). At higher cell densities a more complex relationship is expected due to particle blocking (Dabros and van de Ven, 1982). For example, it was shown that adsorption rate coefficients were a better predictor of microbial transport through a porous medium column than measured parameters of cell size, motility, or hydrodynamics in the column (Camper *et al.*, 1993).

The goal of this research was to determine the adsorption characteristics for healthy and starved bacteria, as well as those with and without motility. The resulting changes in adsorption rate were separated into transport and adsorption processes. Results were obtained for healthy *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* (Mot + and Mot −) grown in a continuously fed stirred tank reactor (CFSTR). In the flow cell 316 stainless steel was used as the substratum. Long term starvation experiments were performed with *P. aeruginosa* and the Mot + strain of *P. fluorescens*.

MATERIALS AND METHODS

Experimental system

The experimental system utilized was developed by Mueller *et al.* (1992) (Fig. 1). The system consists of a flat plate flow cell sufficiently thin to allow direct observation of the attached bacteria by microscopy. A coupon of the material of choice was placed in the flow cell prior to inoculation. In these experiments, glass, polycarbonate, and stainless steel coupons that had been polished with 0.3 μm alumina (Buehler Alpha Micropolish II deagglomerated alumina) were used. Surface roughness for the stainless steel coupon was determined from a reflected light scatter distribution using a Rayleigh perturbation relationship (Stover and Serati, 1984). Light scatter was used to calculate a root mean square (RMS) surface roughness value for the stainless steel surface of 121 Å. Cells were pumped through the flow cell, and the accumulation of bacteria measured nondestructively in real time by recording images at defined time intervals. The inoculum was prepared in a CFSTR with glucose as the carbon source (40 mg l^{-1} as carbon). Growth in all experiments was carbon limited and pH was phosphate buffered (pH 6.8). Temperature was maintained at 22 ± 1°C with a dilution rate of 0.2 h^{-1}.

Because of a relatively high cell concentration in the CFSTR, the effluent was diluted with buffer solution of the same composition as the growth medium without glucose addition. The diluted CFSTR effluent was pumped at a constant flow rate of 1.2×10^{-4} $m^3 h^{-1}$ and a mean bulk water velocity of 2.75×10^{-2} m s^{-1} through the flow cell. These conditions corresponded to a laminar flow regime ($Re = 1.4$) at a shear stress at the coupon surface of 0.75 N m^{-2}. The stainless steel surface was monitored continuously using reflective light and a Nomarski lens, while transparent coupons were monitored by light microscopy. Images were captured and the accumulation of cells on the surface quantified by an image analysis system. The use of image analysis to measure bacterial surface colonization is described in more detail by Mueller *et al.* (1992). All surfaces used for colonization experiments were washed in a sequence of sterile water, acetone, a 70% ethanol/water solution, and sterile water again (Mueller *et al.*, 1992).

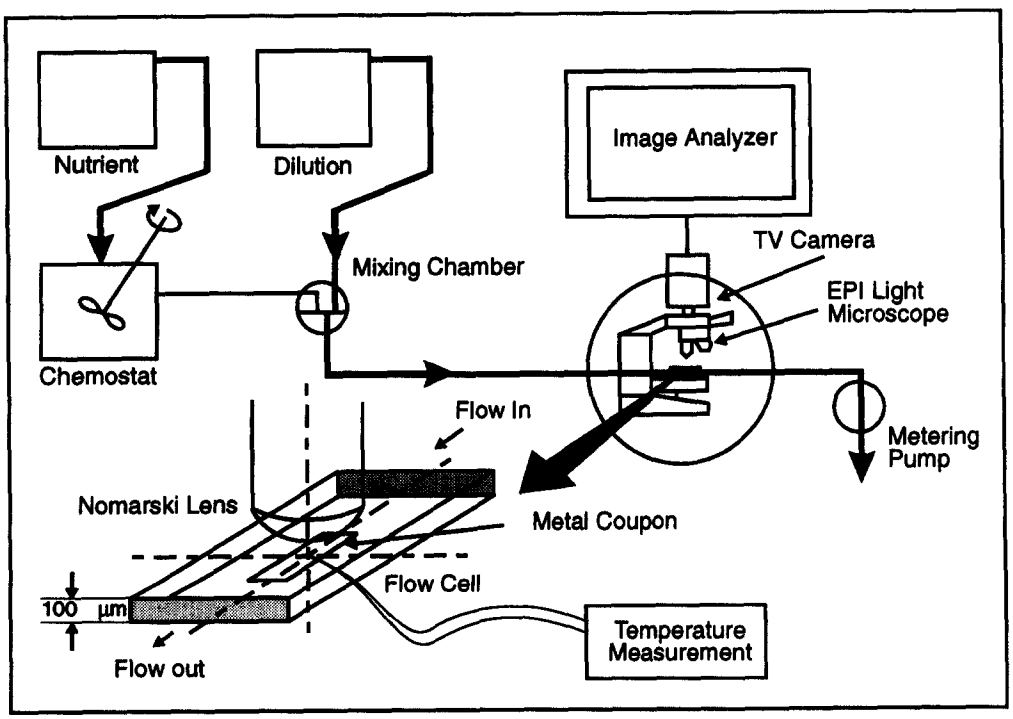

Fig. 1. Schematic diagram of the experimental system, consisting of a CFSTR, a parallel flow-through cell, and a microscope equipped with image analysis.

Analytical methods

Total cell counts. Bacterial numbers in the CFSTR and flow cell effluent were determined by an acridine orange direct count. Bacteria in the fluid samples were fixed in 2% formalin, homogenized, and stained with 0.01% acridine orange for 30 min. Organisms were collected on 0.2 μm pore size black polycarbonate filters and counted using image analysis at 1000 × total magnification (Hobbie *et al.*, 1977). Numbers obtained from 10 fields per sample were averaged.

Viable cell counts. Viable cell numbers were determined in triplicate on R2A agar (Difco) by the spread plate technique after appropriate dilution in sterile buffer. Plates were incubated for 2 days at room temperature and the results recorded as the arithmetic mean of the three replicate values.

Organic carbon. Dissolved organic carbon was determined by filtering samples through a PTFE membrane (pore size 0.2 μm) pre-rinsed 10 × with dilute phosphoric acid. The carbon content of the filtrate was measured using a Dohrmann Model DC 80 total organic carbon analyzer. Triplicate measurements were made for each evaluation and the average value reported.

Organisms

Pseudomonas aeruginosa was isolated from a pipeline environment and characterized by Mueller *et al.* (1992) and Robinson *et al.* (1984). *Pseudomonas fluorescens* strain CC-840406-E (Mot +) and a nonmotile transposon mutant (Mot −) were provided by Darren Korber, University of Saskatoon, Saskatchewan, Canada. The Mot − strain was found to lack a flagellum by transmission electron microscopy analysis (Korber *et al.*, 1989). There was no significant difference between the growth rate of *P. aeruginosa* and *P. fluorescens* of the Mot + parent and the Mot − mutant in batch cultures ($\mu_{max} = 0.45\ h^{-1}$), (Korber *et al.*, 1989).

The two organisms (*P. aeruginosa* and Mot + *P. fluorescens*) were prepared for starvation experiments by terminating the CFSTR influent while maintaining aeration and mixing. Organisms starved for up to 50 days were utilized in the experiments. Cell size and concentration were monitored over time.

Estimation of cell size distribution

Cell sizes were determined in the bulk water and on the substratum by using image analysis with $n > 40$ for each cell size distribution reported. Cell size distributions on the substratum were determined each time the cell surface density on the substratum was measured.

Estimation of cellular motility

The substratum was exposed to the diluted cell suspension containing 10^6–10^7 CFU ml^{-1} until a cell surface density of approximately 4000 CFU mm^{-2} was reached. After initial adsorption occurred, the flow over the substratum was terminated. Within 5–10 min, most of the initially adsorbed cells became motile on the surface. The surface was monitored continuously at a magnification of 1500 × and recorded by a high resolution video camrecorder. The TV screen was calibrated by recording a calibration slide at the same magnification in the horizontal and vertical directions. The random free runs were measured by marking traveling phase (free run) and twiddling phase (change in traveling direction). When replaying the tape in slow motion with time control, the cellular swimming speed for a free run could be estimated. The same procedure was used to test Mot − cells for motility.

Measurement of rates

Adsorption/desorption. These rates were obtained by counting individual bacteria on the surface over time. Cell locations and size were also determined. Measurements were taken at 10 min time intervals. A linear regression of the number of cells adsorbing (new to an area) or desorbing (absent from an area previously recorded) versus time yielded estimates for the adsorption and desorption rate.

Growth/erosion. Colonization of the surface was allowed to proceed until a density of 2000–3000 cells mm^{-2} was reached. Bulk water flow was then switched from diluted CFSTR effluent to the sterile CFSTR influent. The rate of growth was determined by following division of individual cells with time. Simultaneously, erosion was obtained by observing the number of bacteria leaving immobilized colonies. Specific growth rate, doubling time, and erosion rate were determined by regression analysis. Growth or erosion was not measured during the starvation experiments.

RESULTS

Results were obtained for three different bacterial strains (*P. aeruginosa* and *P. fluorescens* Mot + and Mot −) and for three different materials (316 stainless steel, polycarbonate, and glass). Adsorption of healthy cells of *P. aeruginosa* and *P. fluorescens* Mot + growing at a dilution rate of $0.2\ h^{-1}$ was compared to that of starved cells of either strain. Adsorption of the motile strain was also compared to the nonmotile counterpart.

Substrata colonization of three Pseudomonas *strains*

Figure 2 is a cumulate plot of adsorption, desorption, growth, and net accumulation onto 316 stainless steel over 300 min for *P. aeruginosa* growing on glucose at a growth rate of $0.2\ h^{-1}$. During this time period the major factor contributing to cell accumulation on the stainless steel substratum was adsorption. Net growth (growth rate less rate of erosion) was observed after 200 min. The cellular growth and erosion rate for *P. aeruginosa* on stainless steel was 0.33 ± 0.06 and $0.09 \pm 0.08\ h^{-1}$, respectively.

Comparisons were made for rate coefficient of adsorption, the probability of desorption, and the sticking efficiency for the three healthy species. The specific rate coefficient for adsorption was significantly lower for *P. fluorescens* (Mot + and Mot −) than for *P. aeruginosa* regardless of the substratum (Table 1). The Mot − *P. fluorescens* had the lowest specific rate coefficient for adsorption on all substrata. In contrast, the lowest sticking efficiency was seen with the Mot + *P. fluorescens*. The substratum as well as the bacterial strain influence the kinetic parameters of adsorption. For Mot − *P. fluorescens* the specific rate coefficient for adsorption, desorption, and sticking efficiency were highest for the stainless steel substratum. Glass had the lowest specific rate coefficient for adsorption and sticking efficiency, and an intermediate desorption rate.

Effect of starvation

Dwarfing and fragmentation. The CFSTR nutrient supply was cut off after steady state conditions were reached to observe cellular colonization by *P. aeruginosa* and *P. fluorescens* in oligotrophic environ-

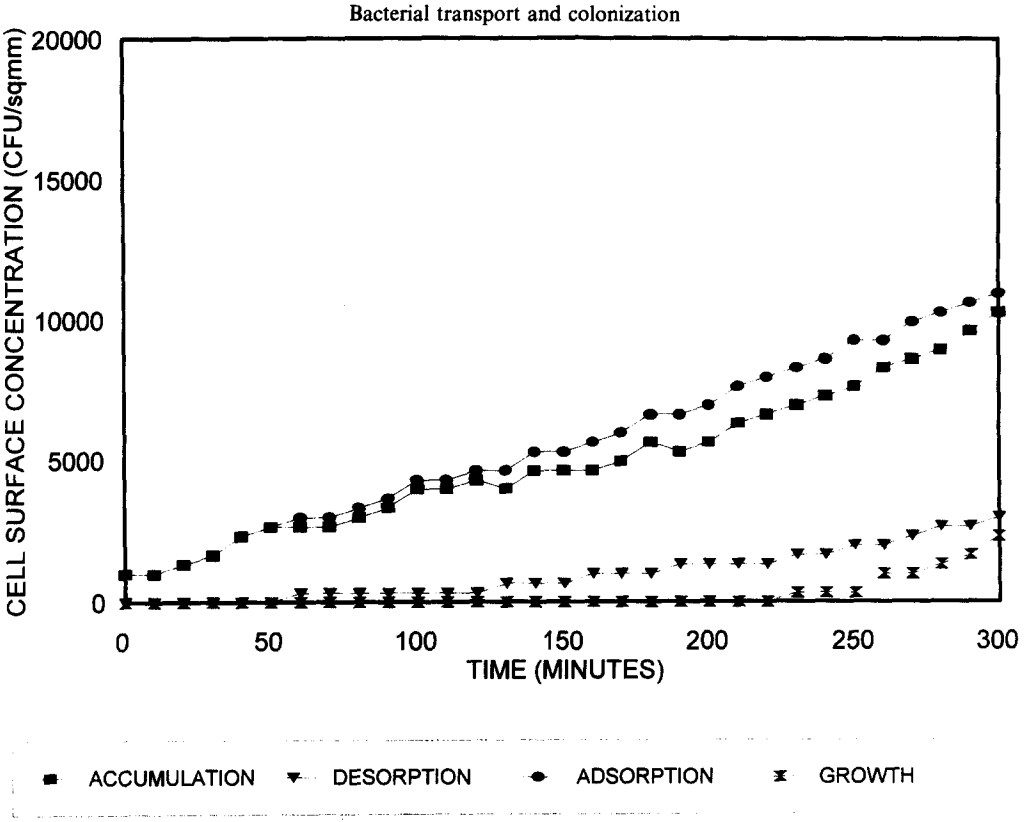

Fig. 2. Progression of typical cell accumulation on 316 stainless steel at a bulk water cell concentration of 1.1×10^6 cells ml^{-1}. Accumulation is displayed as net result of adsorption, desorption/erosion, and growth.

ments. Stirring and aeration in the reactor did continue and subsequently the CFSTR was changed to a batch stirred tank reactor (BSTR). Since the BSTR has no influent or effluent, water flow to the flow cell was achieved by water depletion in the reactor vessel. Because of the high cell concentration in the BSTR, the out-take was diluted with buffer solution of the same composition as the growth medium without glucose addition. The cell size distribution and the cell concentration were monitored over time. Immediate responses of both species to starvation were size reduction (dwarfing) and an increase in cell number (fragmentation). For *P. aeruginosa*, total and viable cell concentrations increased after 24 h and plateaued until 8 days of starvation (Fig. 3). From 8 to 12 days, the cell concentration decreased continuously. The average size, determined as cell surface area, was plotted as a function of starvation duration for *P. aeruginosa* (Fig. 4). The average size of healthy cells was $0.91 \pm 0.14\ \mu m^2$. After 24 h of starvation, the average cell size decreased to $0.58 \pm 0.02\ \mu m^2$. For longer durations of starvation the average size remained relatively constant (range 0.45–0.58 μm^2). The average cell size for healthy *P. fluorescens* was $0.86 \pm 0.12\ \mu m^2$ which decreased to $0.54 \pm 0.03\ \mu m^2$

Table 1. Summary of the kinetic results for *P. aeruginosa* and *P. fluorescens* (Mot + and Mot −) for three substrata (bacteria were grown at a dilution rate of $0.2\ h^{-1}$)

Bacterial strain	Substratum	K_a ($mm\ h^{-1}$)	$1 - \alpha$ (–)	Φ (–)
P. fluorescens	(Mot +)			
	316 stainless steel	0.30 ± 0.03	0.61 ± 0.20	0.0036 ± 0.0006
	Glass	0.25 ± 0.01	0.41 ± 0.25	0.0029 ± 0.0003
	Polycarbonate	0.23 ± 0.02	0.20 ± 0.22	0.0028 ± 0.0002
P. fluorescens	(Mot −)			
	316 stainless steel	0.18 ± 0.04	0.84 ± 0.11	0.48 ± 0.03
	Glass	0.09 ± 0.02	0.23 ± 0.03	0.17 ± 0.06
	Polycarbonate	0.15 ± 0.02	0.03 ± 0.01	0.33 ± 0.01
P. aeruginosa				
	316 stainless steel	1.84 ± 0.22	0.30 ± 0.18	0.022 ± 0.002
	Glass*	1.14	0.62 ± 0.18	0.018

K_a = specific rate coefficient of adsorption.
$1 - \alpha$ = probability of desorption.
Φ = sticking efficiency.
*From Mueller *et al.* (1992).

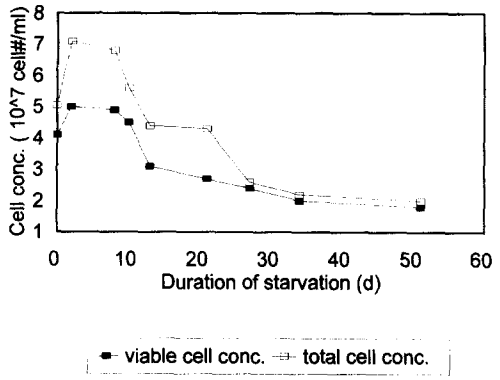

Fig. 3. Total and viable suspended cell concentration of *P. aeruginosa* plotted versus duration of starvation.

Table 2. Motility and diffusivity results for all bacterial strains

Bacterial strain	v_r (μm s^{-1})	d_r (μm)	D_c (mm^2 s^{-1})
P. aeruginosa	55.8*	50–85*	1.0×10^{-3}
P. aeruginosa (starved 216 h)	100–200	33 ± 11	1.3×10^{-3}
P. fluorescens (Mot +)	60 ± 34	60 ± 21	1.2×10^{-3}
P. fluorescens (Mot +, starved 200 h)	100–200	42 ± 16	2.1 ± 10^{-3}
P. fluorescens (Mot −)	0	0	3.9×10^{-7}

v_r = swimming speed.
d_r = length of free run.
D_c = calculated diffusivity.
*From Mueller *et al.* (1992).

after 8 days of starvation. For both species under starved conditions the size decrease was statistically significant, based on a 95% confidence interval.

Cellular diffusivity. Cellular motility was of interest due to the dependency of diffusivity on motility and cell size. Swimming speed and the length of runs was measured for healthy and starved *P. aeruginosa*, healthy Mot + and Mot − *P. fluorescens*, and starved Mot + *P. fluorescens*. For both strains tested the starved bacteria responded with a substantial increase in swimming speed and a slight reduction in the length of random free runs when compared to healthy cells (Table 2). Starved free runs, as determined from more than 47 observations for each cell type, were 33 ± 11 μm and 42 ± 16 μm for *P. aeruginosa* and *P. fluorescens*, respectively. The cellular swimming speed for either species was increased by up to four-fold over healthy cells. Using these experimentally determined motility parameters, diffusivities were calculated. Both starved species exhibited higher diffusivity values than those for healthy cells. The average diffusivity value for *P. aeruginosa* after 8 days of starvation was $1.3 \pm 0.8 \times 10^{-3}$ mm^2 s^{-1}, which was not significantly different ($p = 0.05$) than the diffusivity value reported for healthy cells ($D_c = 1.0 \times 10^{-3}$ mm^2 s^{-1}). For starved *P. fluorescens* the diffusivity value ($D_c = 2.1 \pm 0.5 \times 10^{-3}$ mm^2 s^{-1}) was significantly higher ($p = 0.05$) than for healthy cells ($D_c = 1.2 \times 10^{-3}$ mm^2 s^{-1}).

Rate of cellular adsorption to a substratum. Adsorption/desorption of starved *P. aeruginosa* cells was determined as a function of starvation duration. Zero time values are for healthy cells grown at a dilution rate of 0.2 h^{-1}. The specific rate coefficient for adsorption, detachment, and sticking efficiencies decreased until 8 days of starvation (Table 3). The specific rate coefficient for adsorption then remained relatively constant ($K_a = 0.4$–0.6 mm h^{-1}), as did the probability of desorption and the sticking efficiency.

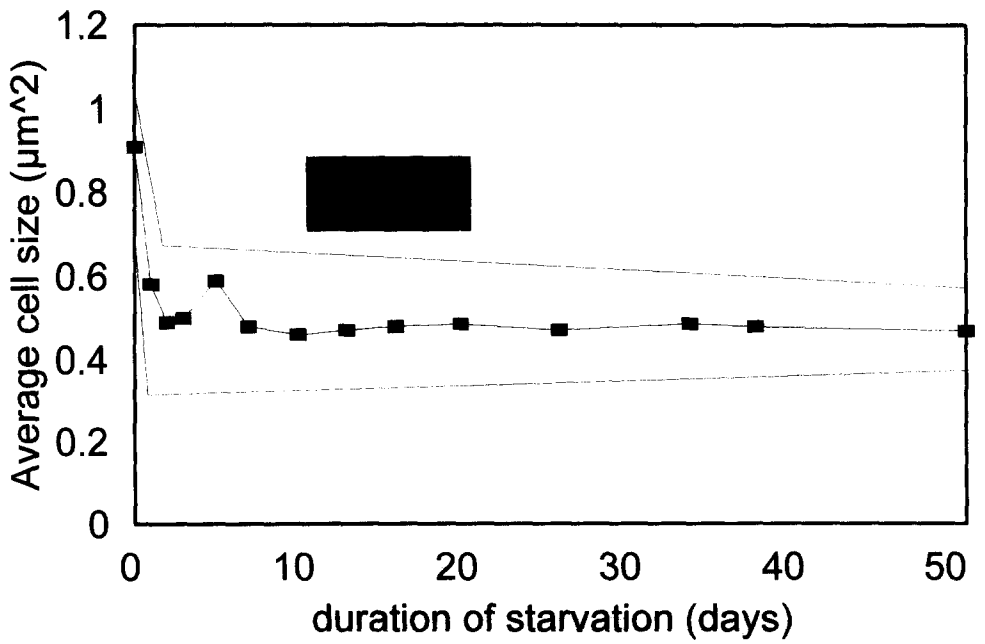

Fig. 4. Cell size analysis performed on suspended *P. aeruginosa* cells during starvation. The average cell sizes are plotted versus duration of starvation.

Table 3. Kinetic results for starved *P. aeruginosa* on 316 L stainless steel

t_{st} (h)	K_{av} (mm h^{-1})	$1 - \alpha$ (–)	Φ (–)
0	1.56 ± 0.34	0.28 ± 0.02	0.019 ± 0.005
72	1.21 ± 0.29	0.17 ± 0.03	0.010 ± 0.002
216	0.90 ± 0.17	0.10 ± 0.03	0.007 ± 0.001
348	0.95 ± 0.16	0.07 ± 0.04	0.008 ± 0.002
504	0.54 ± 0.16	0	0.004 ± 0.002
(648–1224)*	0.47 ± 0.15	0.08 ± 0.05	0.004 ± 0.001

t_{st} = duration of starvation prior to addition to flow cell.
K_{av} = averaged specific rate coefficient of adsorption.
$1 - \alpha$ = probability of desorption.
Φ = sticking efficiency.
*The values for 648, 816, 936, and 1226 h of duration of starvation were averaged.

The rate of adsorption/desorption for starved Mot + *P. fluorescens* was observed at one specific time (8 days of starvation) and compared to cells at zero time. The cell concentration was constant during each adsorption experiment, with values between 10^6 and 10^7 cells ml^{-1}. The specific rate coefficient for adsorption increased significantly when this organism was starved. Healthy, growing cells adsorbed with $K_a = 0.30$ mm h^{-1} and a probability of desorption of 61%. The value for starved cells increased to 1.08 mm h^{-1} and the probability of desorption was zero. In contrast, the sticking efficiency was not substantially different after starvation (0.004 ± 0.002 before versus 0.009 ± 0.003 after). Therefore, increased transport rather than sticking efficiency was responsible for the increased specific rate coefficient for adsorption. In all starvation experiments, no growth or cell division was observed on the substratum for either species during the time period monitored.

DISCUSSION

Simulation of surface colonization of a mixed population

The rate of cell accumulation on a substratum can be expressed as a function of net adsorption rate and net growth rate:

$$dX''/dt = \alpha K_a X_b' + (\mu_s - K_e)X''. \quad (7)$$

Derivation of this equation is described in detail in Mueller *et al.* (1992). This model was used with measured parameters to simulate the accumulation of the bacterial strains on a substratum.

Different cells were shown to exhibit different surface colonization characteristics. Even at the same growth state $\mu = 0.12\ h^{-1}$, *P. aeruginosa* cells colonized the surface much more rapidly than *P. fluorescens* cells. This is illustrated in Figs 5 and 6, where the cell surface colonization was simulated on the basis of the experimentally determined kinetic data. After 500 min five times more *P. aeruginosa* cells had accumulated on the substratum surface than *P. fluorescens*. Hence, the latter may be "out-competed" in a specific system when both bacteria are initially present in suspension. Differences in cellular surface colonization have been shown to be important in cell separation/purification techniques (Ross and Hjortso, 1989) and could possibly affect the structure and composition of the entire biofilm formed on a surface (Mueller, 1994).

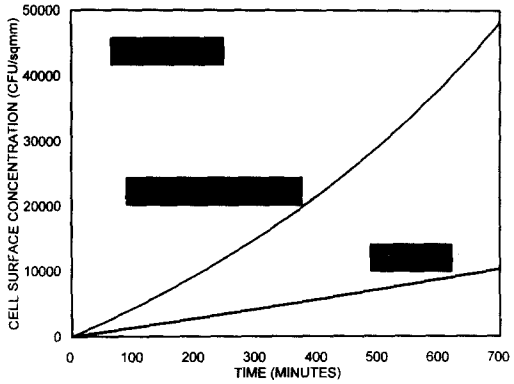

Fig. 5. Simulation of cell accumulation of *P. aeruginosa* on stainless steel for healthy cells growing at a growth rate of 0.12 h^{-1} and under nutrient depletion, where starvation may occur. Shear stress at the substratum surface and the suspended cell concentration were kept constant during the simulation at 0.75 N m^{-2} and 2×10^6 cells ml^{-1}, respectively. Simulated cellular accumulation is plotted versus time of exposure to a stainless steel substratum.

In very low nutrient environments where starvation is occurring, the adsorption characteristics of a specific cell can be significantly different from healthy cells of the same species. *P. aeruginosa* cells exhibited a decrease in their surface colonization rate by about 50% (Fig. 5), whereas *P. fluorescens* increased their rate of adsorption almost four-fold (Fig. 6). Hence, when comparing the same species at different environmental conditions, such as low nutrient or nutrient-depleted environments, where starvation may occur, the two species tested showed the reverse colonization behavior than at high nutrient

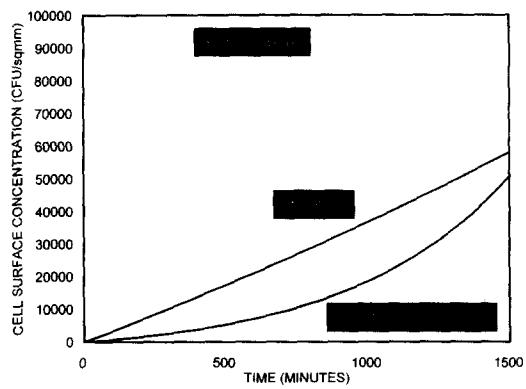

Fig. 6. Simulation of cell accumulation of *P. fluorescens* on stainless steel for healthy cells growing at a growth rate of 0.12 h^{-1} and under nutrient depletion, where starvation may occur. Shear stress at the substratum surface and the suspended cell concentration were kept constant during the simulation at 0.75 N m^{-2} and 2×10^6 cells ml^{-1}, respectively. Simulated cellular accumulation is plotted versus time of exposure to a stainless steel substratum.

environments (Figs 5 and 6). In the case of starvation, *P. fluorescens* colonized surfaces at twice the rate of *P. aeruginosa* when all other parameters including suspended cell concentration, flow rate, and the resulting shear stress at the substratum surface were kept constant. Hence, the environmental conditions as well as the species specific growth and attachment kinetics could determine the competition outcome in a biofilm system.

Comparison of Mot + and Mot − Pseudomonas fluorescens

Cellular surface colonization by motile and nonmotile cells was compared using *Pseudomonas fluorescens* as a Mot + and Mot − strain. For this comparison, 316 stainless steel, glass, and polycarbonate were used as substrata. The Mot − mutant did not possess a flagellum, as the parent Mot + strain did. The probability of desorption was similar for the two strains on all the investigated substrata. The observed adsorption coefficient of motile cells was twice as high as that for nonmotile cells (Table 1). Korber *et al.* (1989) reported four-fold increase in recolonization rate of motile cells of *P. fluorescens* compared to the Mot − mutants. However, there is a much larger difference between the transport properties of motile cells and nonmotile cells. The difference in diffusivity is almost four orders of magnitude. A higher value for sticking efficiency for the Mot − mutant as compared to the Mot + cells may indicate a transport limitation rather than a limitation in adsorption for the Mot − cells. This finding might exclude the possibility that the cell flagellum is of major importance for adsorption mechanisms on the substratum (Kefford and Marshal, 1984; Fletcher, 1980). However, the cellular adsorption rate and, therefore, the rate of cell colonization on a substratum is largely dependent on the cell transport rate from the bulk water to the solid–water interface. Hence, flagellated cells adsorb and accumulate on a substratum at a higher rate than unflagellated cells.

Starvation and microbial surface colonization

Immediate responses of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* to starvation were size reduction (dwarfing), and an increase in cell number (fragmentation). Kjelleberg and Hermansson (1984) also reported fragmentation and continuous size reduction (dwarfing) of the suspended organisms as a response to the initial phase of starvation (4 h). The reduction in cell size may indicate an adaptation of the cells to the low nutrient environment by increasing their surface/volume ratio and therefore their efficiency in nutrient assimilation.

An increased swimming speed was observed for both species under starvation. The increase in swimming speed could be caused indirectly by the size reduction the cells underwent during the first 24 h of starvation. The increased cell motion resulted in a significant increase in diffusivity for *P. fluorescens*. The increase in diffusivity for *P. aeruginosa* was statistically not significant. Starved cells of *P. fluorescens* had superior transport properties over healthy, growing cells of the same strain.

Sticking efficiency was calculated from transport properties and the specific rate coefficient for adsorption (Escher and Characklis, 1990). Sticking efficiency, the specific rate coefficient for adsorption, and the probability of desorption decreased with duration of starvation of *P. aeruginosa*. Sticking efficiency decreased proportionally to the specific rate coefficient for adsorption, since diffusivity (i.e. transport rates) did not change significantly for this organism.

When adsorbed to the substratum, the probability of desorption decreased significantly for starved cells. This can be explained by an increase in substrate flux for adsorbed cells. Therefore it would be energetically not favorable for the adsorbed cells to desorb from the substratum. When *P. fluorescens* cells were starved the rate of adsorption increased four-fold. The difference in sticking efficiency for *P. fluorescens* was statistically not significant. Hence the higher rate of cellular adsorption of *P. fluorescens* was due to an increased cellular transport rate to the solid–liquid interface.

Kjelleberg *et al.* (1982) reported that the irreversible adsorption of *Pseudomonas* sp. strain S9 (hydrophobic) and *Vibrio* sp. strain DW1 (hydrophilic) increased during 4 h of starvation. Kjelleberg (1984) did a similar experiment with seven marine isolates and found a small increase in irreversible adhesion after 5 h, followed by a small decrease after 22 h of starvation of six strains. For one strain, the irreversible binding remained constant. This indicates a cell type specific change in adsorption/transport rate, as we found with the two *Pseudomonas* species we tested.

Marshall (1979) reported regrowth to a normal size of small, dwarf-like bacteria within 12–20 h after surface colonization. Attachment of microorganisms to carbon particles in a low nutrient environment was shown to prevent a reduction in cell size (Davies and McFeters, 1988). The authors stated that this phenomenon indicated a higher nutrient availability at the solid–liquid interface than in the bulk water, and that the bacteria attached to carbon particles in low nutrient drinking water have an enhanced survival advantage.

Transport phenomena in cellular colonization

As our results indicated, transport has a major effect on microbial deposition rates. Motile cells exhibit superior transport properties and subsequently colonize at a faster rate than nonmotile species. Korber *et al.* (1990) found a four-fold increase in attachment rate for *P. fluorescens* (Mot +) compared to an otherwise identical nonmotile mutant. However, this higher rate of

attachment does not necessarily represent a higher sticking efficiency, if one is capable of separating the transport process from the actual process of cellular adsorption to the surface. Korber's results may also reflect a difference in diffusivity or transport properties. Many starvation studies reported a size reduction of the cells after a short period of starvation. This size reduction may have an effect on the swimming speed of these cells and therefore the diffusivity should be treated as a function of the cell microenvironment. The actual difference in observed adsorption rate may be caused by a change in transport and not only be due to changes in the cell wall physiology or other cellular attachment mechanisms.

CONCLUSIONS

A technique was developed to measure initial cell colonization on reflective metal surfaces at varying environmental conditions, based on previous work (Mueller *et al.*, 1992). Experiments were conducted with *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* as a Mot + and a Mot − strain. The following conclusions have been derived from this work.

Resulting from superior transport properties, flagellated cells of *Pseudomonas fluorescens* adsorb and accumulate on the substratum at a higher rate than unflagellated cells.

Nutrient conditions in the bulk water not only influence the growth of suspended and attached cells but also influence cellular transport to the solid–water interface, as well as the rate of adsorption and desorption onto the substratum. Starved cells of *P. aeruginosa* and *P. fluorescens* exhibited increased cell motility by size reduction. In low nutrient environments attachment might be used as a strategic survival mechanism of organisms. This is one way to increase the flux and availability of nutrients to the cells. Size reduction of planktonic organisms was reported as another mechanism for improvement of nutrient availability.

Cellular transport is a crucial process in microbial colonization, and may be limiting the overall rate of surface colonization. This phenomenon was demonstrated for a motile and nonmotile mutant of the same strain. Hence, diffusive, advective, and hydrodynamic properties of a specific system cannot be neglected if we are to try to understand and simulate initial events of microbial colonization.

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