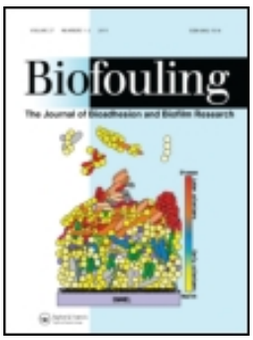

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Short Communication

DEPOSITION OF BACTERIAL CELLS ONTO GLASS AND BIOFILM SURFACES

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Deposition rates of *Pseudomonas putida* and *Hyphomicrobium* ZV620 onto glass and biofilm surfaces were quantified. Both species deposited to glass at a much slower rate than to biofilm. A definite bias by depositing cells for biofilms of their own species was evident in the highest attachment rates observed in this study.

KEY WORDS: bacterial deposition, biofilm, attachment, substratum.

INTRODUCTION

A biofilm is a surface accumulation of microorganisms, frequently characterized by large amounts of organic polymers of microbial origin that bind cells and other organic and inorganic materials together and to the substratum. A biofilm community begins initially when a clean surface is exposed to an aqueous environment. Inevitably, microbes become associated with the surface, adsorb, replicate, and produce exopolymers while metabolizing ambient nutrients. As the biofilm gel matrix accumulates, it acts to trap nutrients and to attract other microbial participants into the biofilm community. Thus, the microbial activity and diversity of a dynamic biofilm community is dependent upon cell growth at local nutrient conditions and the dynamic (two-directional) exchange of cells between the biofilm and fluid phase (Characklis, 1984).

While sufficient information regarding the removal of cells and biomass from either a substratum or biofilm is beginning to emerge (Powell & Slater, 1983; Characklis, 1984; Escher, 1986), very few data exist regarding the deposition of cells onto an existing biofilm. In most research on microbial cell deposition, attention has been directed towards the initial events governing cell adsorption to a clean substratum (Powell & Slater, 1983; Escher, 1986; McEldowney & Fletcher, 1987; Sjollema *et al.*, 1988). In research reported here, the deposition rates of two pure bacterial cultures onto clean glass are determined and compared to the rates of cell attachment of the two species onto established biofilms of both species. These rates are also compared to ascertain whether the presence of a fully developed biofilm affects the deposition or capture of suspended bacteria.

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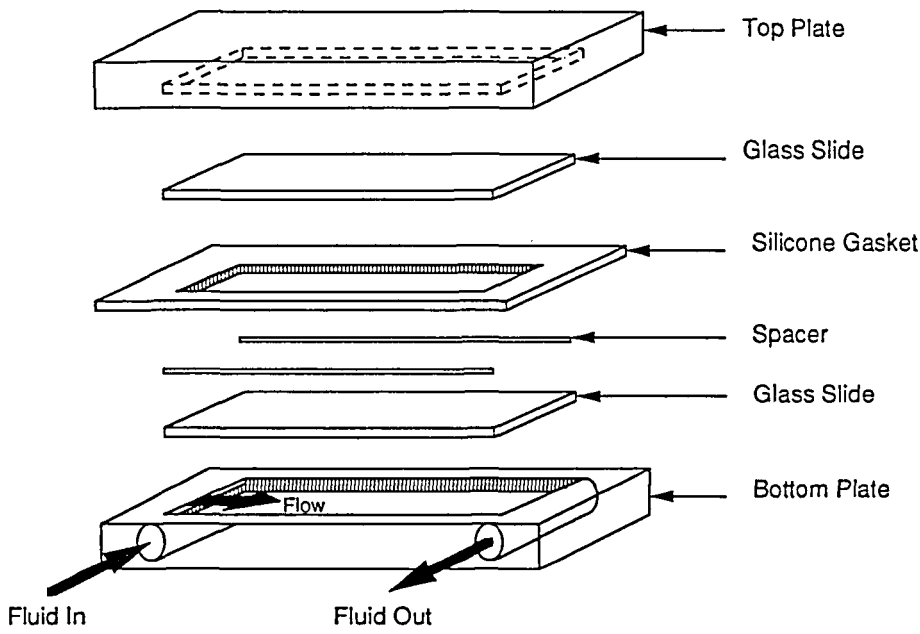

Fig. 1 Flow Cell Reactor.

MATERIALS AND METHODS

Pseudomonas putida ATCC 11172 and *Hyphomicrobium* sp. ZV620 were the species used in this study. *P. putida* cells were grown in a glucose medium (Banks & Bryers, 1991) in suspended culture from freeze-dried samples. *Hyphomicrobium* sp. cultures were resurrected from slants and grown as suspended cultures in methanol medium (Grazer-Lambert *et al.*, 1986). For the following experiments, each species was grown as a suspended batch culture to early stationery phase as detected by optical density measurements.

Total bacterial counts in suspension were determined by epifluorescence microscopy as described by Hobbie *et al.* (1977). Scintillation counts were measured with a Packard Tri-Carb 1900 CA liquid scintillation counter, using Filter-Count (Packard, Downers Grove, IL) as the fluor. Biofilm thickness was determined using the micrometer on the fine focus adjustment of a Reichert-Jung Micro-Star IV (Reichert-Jung, Cambridge, MA) at 10× magnification (Bakke & Ollsen, 1986). Glucose concentrations were determined enzymatically using a glucose oxidase assay (Kit #510) from Sigma Biochemicals (St Louis, MO). Methanol concentrations were measured using a Shimadzu GCA-9 gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD) equipped with an 80/100 Carbopack C-0·1% SP 1000 column (Supelco Incorporated, Bellefont, PA) and flame ionization detector.

All deposition studies were carried out in a rectangular duct flow cell reactor depicted in Figure 1 and characterized in Table 1. Top and bottom halves of the flow cell, constructed of transparent plexiglass, were designed to accept standard glass microscope slides within two indentations, thus forming a rectangular duct with glass as the substratum. Prior to each experiment, the glass slides were prepared by washing

Table 1 Operating details of the rectangular flow cell reactor

Internal Dimensions	
Channel width (cm)	2.0
Channel length (cm)	7·5
Channel height (cm)	0·5
Internal surface area (cm^2)	39·5
Volume (cm^3)	7·5
Constant Operating Conditions	
Volumetric flow rate to flow cell ($cm^3 \cdot min^{-1}$)	0·2
Fluid velocity in flow cell ($cm \cdot s^{-1}$)	$3{\cdot}2 \times 10^{-2}$
Temperature (°C)	20
Biofilm Growth Conditions	
Glucose concentration ($mg \cdot l^{-1}$)	500
Methanol concentration ($mg \cdot l^{-1}$)	533
Particle Deposition Conditions	
Suspended *P. putida* concentration ($cells \cdot ml^{-1}$)	$1\text{–}2 \times 10^8$
Suspended *Hyphomicrobium* sp. concentration ($cells \cdot ml^{-1}$)	$4\text{–}5 \times 10^7$

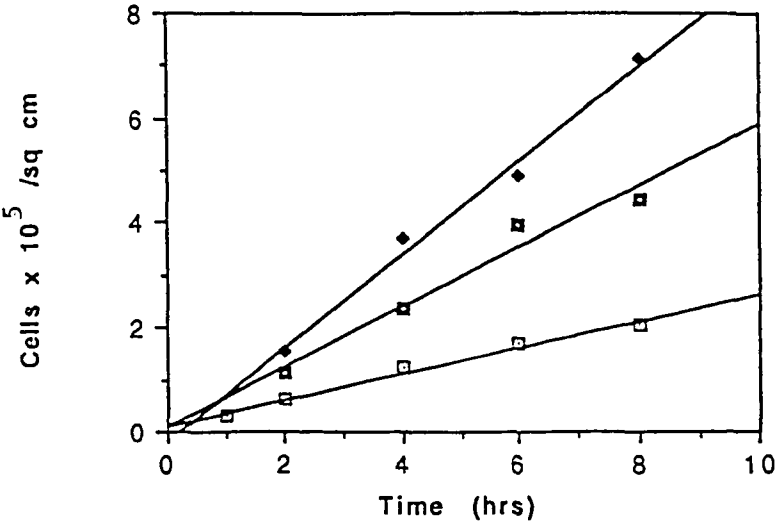

Fig. 2 Deposition of *Pseudomonas putida* cells onto glass surface ⊡; *P. putida* biofilm ◆; *Hyphomicrobium* sp. biofilm ◘

with 10% HCl, and then rinsing with filtered, autoclaved water. The reactor base was cleaned by soaking in 70% ethanol and rinsing with filtered, autoclaved water.

Pure culture biofilms of either *P. putida* or *Hyphomicrobium* sp. were initiated by first inoculating the flow cell reactor with a flowing suspension of cells under the conditions listed in Table 1. After 6 h, feeding of the bacterial suspension was terminated, the system flushed of all suspended cells, and a sterile nutrient solution (Grazer-Lambert *et al.*, 1986; Banks & Bryers, 1991) delivered to the reactor under the same flow

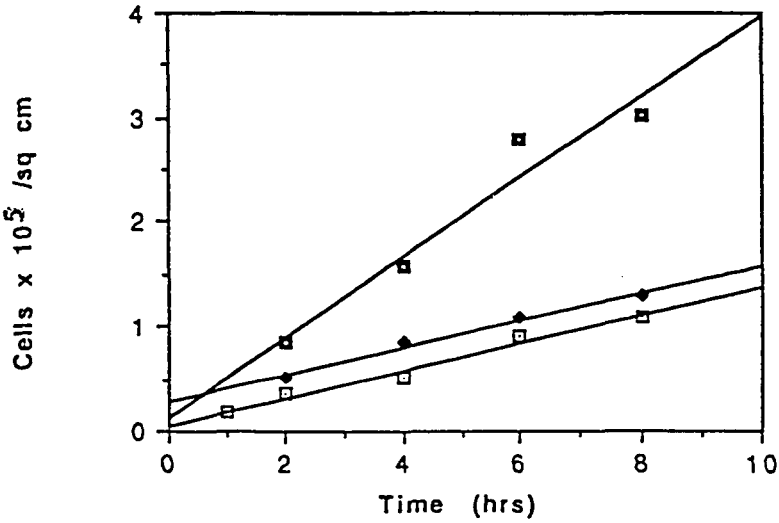

Fig. 3 Deposition of *Hyphomicrobium* sp. cells onto glass surface ⊡; *P. putida* biofilm ◆; *Hyphomicrobium* sp. biofilm ■

Table 2 Rate of bacterial cell deposition and sticking efficiency

Depositing species	*Recipient surfaces*	*Observed rate of deposition/attachment (cells·cm^{-2} s^{-1})*	*Particle flux (cells·cm^{-2} s^{-1})*	*Sticking efficiency (%)*
P. putida	glass	7·0	150	4·7
Hyphomicrobium	glass	3·6	45	8·0
P. putida	*P. putida* biofilm	24·7	150	16·5
P. putida	*Hyphomicrobium* biofilm	16·1	150	10·7
Hyphomicrobium	*P. putida* biofilm	3·6	45	8·0
Hyphomicrobium	*Hyphomcirobium* biofilm	10·6	45	23·6

conditions as described above. Biofilm was allowed to accumulate to a thickness of approximately 90 μm prior to the cell attachment studies.

Cells were radiolabeled (McEldowney & Fletcher, 1987) using 0·5 μCi L-[U-^{14}C] glucose (SA 2–10 mCi·mmol^{-1})·ml^{-1} for *P. putida* or methanol (SA 5–20 mCi·mmol^{-1})·ml^{-1} for *Hyphomicrobium* sp. (Sigma Company, St Louis, MO). After labeling, cells were resuspended in biofilm nutrient media. For the biofilm attachment studies, where the depositing cells were the same species as in the biofilm, the labeled cells were separated from the carbon source until immediately before flowing into the reactor to prevent suspended cell growth. Studies performed on suspended cultures indicated that the radiolabel was stable for the prescribed time period. Dilution of radiolabel due to cell replication and subsequent cell removal was assumed to be insignificant. Calibration curves to determine the specific activity of the labeled

cells were prepared to relate disintegrations per minute to cell number (Banks & Bryers, 1991). Radioactively marked cells of each organism were enumerated by epifluorescence cell counts, for both suspended and attached cultures.

Prior to a deposition experiment, two glass slides were scored on the side opposite to that intended as the substratum, then placed in the accommodating indentations in the plexiglass reactor flow cell. The plexiglass halves were sealed together and labeled cells of either species, suspended in nutrient medium, were delivered to the reactor flow cell under the conditions listed in Table 1. After a prescribed exposure time, the reactor was opened and the glass slides removed, rinsed gently with sterile phosphate buffer, broken at each "score", then placed in a glass scintillation vial. Care was taken to handle the slide using only filter paper. Scintillation counts were made of six glass sections and filter paper; and using the calibrations discussed above, attached cell numbers were calculated.

The attachment rate of the labeled bacterial cells onto a biofilm surface was determined as follows. Biofilms of either species were grown in the flow cell to a thickness of 90 μm. Suspensions of radiolabeled cells of either species were then delivered to the rectangular flow cell reactor under the conditions listed in Table 1. After a prescribed period of exposure, the reactor was opened, a 2 cm^2 sample of biofilm scraped from the microscope slide, and resuspended in phosphate buffer. Six biofilm samples were taken for each prescribed time period. Scintillation counts were measured for each sample.

RESULTS AND DISCUSSION

The results shown represent an average of the six samples taken for each time period (C.V. <9%). Figures 2 and 3 illustrate the results for *P. putida* and *Hyphomicrobium* sp. depositing onto a clean glass surface, a *P. putida* biofilm, and a *Hyphomicrobium* sp. biofilm. These results indicate that the accumulation of radiolabeled cells was independent of the number of cells present on the surface. Consequently, rates of deposition were calculated directly as the slopes of the lines shown in Figures 2 and 3. These calculated rates are summarized in Table 2.

Table 2 also provides estimates of sticking efficiency, the observed rate of cell adsorption or attachment divided by the transport flux of cells to the substratum (Adamczyk & van de Ven, 1981). Rates of deposition and sticking efficiencies are shown to be greater for biofilm surfaces than for glass. In addition, attachment of a species appears to be biased when the biofilm is formed by that species. For *P. putida* cells attaching, the sticking efficiency was higher for a *Hypomicrobium* sp. biofilm than for clean glass surfaces and even higher for the *P. putida* biofilm. *Hyphomicrobium* sp. cells appear to attach equally well to glass and *P. putida* biofilm, but there is a marked improvement in sticking to biofilms of *Hyphomicrobium* sp. cells. This research suggests that sticking efficiency is not only a function of shear stress and bacterial cell type, but also a function of substratum chemistry and morphology.

Acknowledgements

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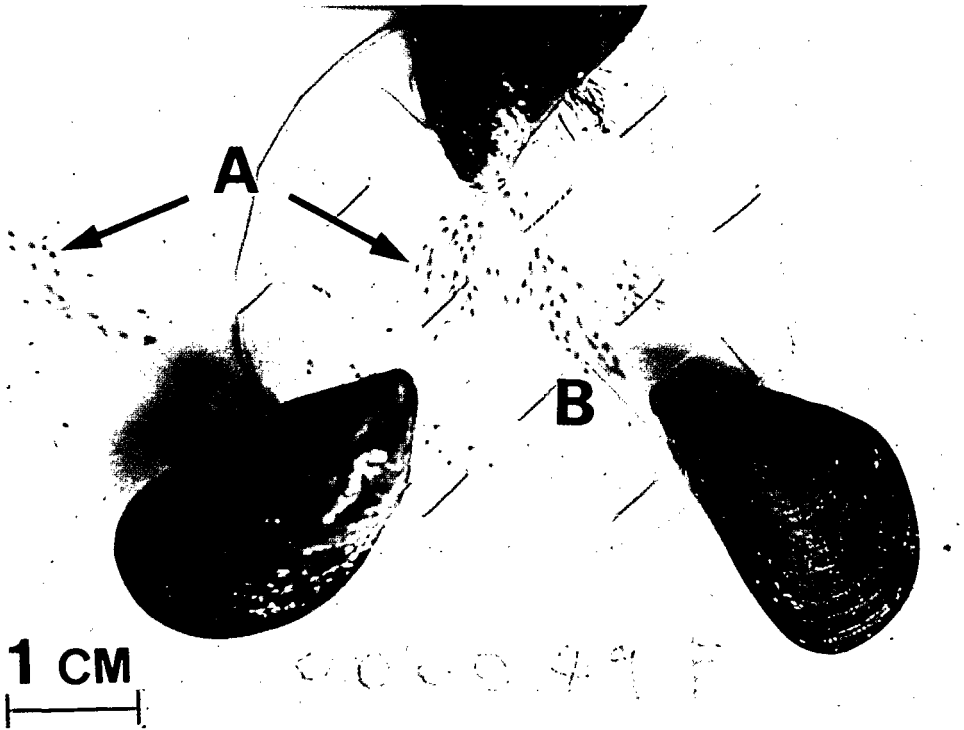

Colour Plate 1. (See Goto *et al.* "Fatty Acids as Antifoulants..." Figure 1)
Fig. 1 Antifouling assay using the blue mussel *Mytilus edulis*. A = byssuses attached to the base, avoiding the sample disk; B = byssuses attached to the sample disk.