Chapter 21

Regulation of External Polymer Production in Benthic Microbial Communities

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INTRODUCTION

Attachment to an inanimate or animate surface is a common mechanism of microorganisms to increase survival. Organisms may form specific consortia with component organisms of different physiological properties to increase the metabolic versatility of the complex. The importance of attachment to specific surfaces is a key feature in the distribution of microorganisms in nature, and the mechanisms and consequences of attachment have been the subject of several intensive studies (14, 23–26, 31, 32, 38, 39). The consequences of specific attachments of microbial parasites to tissues have been recognized from the earliest studies of infectious diseases, and the enormous literature based on these studies is far too complex and extensive to be reviewed here.

In this chapter, only the relatively nonspecific attachment of bacteria to substrata will be considered. It is clear from many studies, for example, the extensive work of Fletcher and co-workers, that the physiological status and metabolic activities of attached and nonattached microorganisms in the same monoculture can be very different (12, 13); thus, making generalizations about the regulation of external polymer production is fraught with risk.

The ubiquity of cell adhesion to a wide variety of surfaces (including other cells) reinforces the fundamental concept that surface contact or proximity must occur prior to attachment. This may represent a phase of relatively nonspecific attachment. This is a prelude to biologically directed adhesion, which may induce a specific response for specific substrata (30) or may evoke a general response. These two stages represent the primary physical attraction, which is reversible, and a secondary, biologically directed adhesion stabilization reaction, which is irreversible (23).

The irreversible step of adhesion often involves the elaboration of extracellular surface structures or polymers. Elucidation of the controls in this complex process is compounded by the diversity of the surface substrates to which the organisms attach, the enormous matrix of organisms that can potentially adhere in monoculture as well as in

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adherent consortia (19), and the multiple mechanisms that organisms use in the attachment process. Cell surface components and structures that are known to influence adhesiveness include thin fimbriae (34), cell surface antigens (34), secreted lipopolysaccharides, and extracellular polysaccharide (glycocalyx) polymers as magnificently illustrated in the work of Costerton et al. (3).

The multitude of responses of different bacteria to changes in the physicochemical parameters of the substrata that influence the specific structure or to changes in the chemistry of extracellular processes responsible for irreversible adhesion strongly indicate that few generalizations are possible and that specific mechanisms for a number of microbes will have to be defined before the fundamental patterns are elucidated. Many of the factors that could influence the specific microbial response in the formation of the irreversible adhesion component will not be considered further, since they complicate the insight into the control of the process. An apparent prerequisite for adhesion in marine systems is the presence in the substratum of carbohydrate-enriched layer scavenged from the bulk liquid phase. Inorganic as well as organic substrates for adhesion are rapidly coated with a layer of adsorbed macromolecules and lipids (11, 14). This is possibly part of the mysterious process of surface conditioning that can either increase or decrease the adhesion of microorganisms to a substratum. The nature of the conditioning film for substrata exposed to the marine environment has so far eluded chemical definition (1). Bacteria also adsorb antibodies and macromolecules from animal hosts, which may or may not promote cell attachment competency (34). This mechanism will not be considered further.

This review will concentrate on methods for studying two responses of specific microorganisms to metal substrata in the marine environment. The two phenomena are the formation of hydrophobic protein fimbriae and the elaboration of acetic extracellular polymer polysaccharides as the biological manifestation of irreversible attachment.

EXOPOLYMER SECRETION

For our purposes adhesion will be defined as the formation of a single interface between two material phases so that mechanical work can be passed through the interface without loss of intimate contact. An adhesive joint is formed from at least two adhesion interfaces with an intervening third phase. By these definitions, an adhesive joint will have two strengths of adhesion and an adhesion interface will have one (15).

Destructive physical and quantitative chemical methods of assessing adhesion involve the examination of the finished bioinorganic interface integrity. Washing with solvents, chemical degradation, and physical stress are the major processes which are coupled to spectroscopic, chromatographic, or gravimetric techniques for quantitative assessment of what remains bound to the test surface. A combination of analytical techniques (gas chromatography [GC], high-pressure liquid chromatography, and gas-chromatographic mass spectrometry [GC-MS]), can be used to elucidate the structure and components forming the biofilm and surface-binding matrix. However, owing to the complexity and diversity of organisms, these techniques do not always provide the definitive results expected, nor do they provide dynamic in situ monitoring of film formation kinetics. Nevertheless, GC-MS techniques (33) have been used to determine the structures of mono-, di-, and polysaccharides, biologically important sugars (deoxyribose, ribose, aminosaccharides, uronic acid [s], phosphorylated sugars, and glycosides). Complex biological saccharides are usually totally or partially hydrolyzed as well as methylated to identify, sequence, and determine the linkages in the polymer (33). Supercritical fluid chromatography (SFC) can

be used to separate the neutral, glycolipid, and polar lipid fractions from various environmental sources. SFC provides the advantage of separating high-molecular-weight, high-boiling-point, and thermolabile compounds at moderate temperatures. With SFC, the high temperature used for volatilization of high-molecular-weight compounds is replaced by fluid density manipulations (18, 35).

Extracellular polymeric substances participate in the formation of microbial aggregates. They are readily detected as capsules and slimes in fungi, bacteria, and algae. Secreted capsular material adheres to the cell wall, whereas slime exists free in the suspending medium. The gel form of the extracellular polymeric substances is hydrated (ca. 98% water); it is dehydrated in the fixation process for electron microscopy and can appear as fibrous strands between the organism and the substrata. Antibody to the extracellular polymeric substances has been used by Costerton et al. (3) before fixation to show the diffuse nature of exopolymer adherent materials. Flocs are larger mats (20 to 200 μm) of mucus- or slime-coated organic particles (16). One means of demonstrating exopolysaccharide function in adhesion has been to use sodium periodate treatment to denature polysaccharide by oxidation and cleavage of vicinal hydroxyl groups (24). This clearly documents the polysaccharide nature of extracellular polymeric substances. Other components possibly involved in attachment include peptidoglycan (minor fraction in gram-negative bacteria and up to 80% in gram-positive bacteria) or teichoic and teichuronic acids (polymers of glycerol and ribitol phosphate with sugar substituents and ester-linked alanine) linked to muramic acid, lipopolysaccharides, and outer membrane proteins (39).

The irreversible step in adhesion of a number of microorganisms involves the elaboration of an extracellular polymer. These substances are primarily polysaccharide with reactive carboxyl, amino, or carbonyl groups on the carbohydrate residues. In eubacteria the uronic acid group seems to provide the acidic properties that characterize these attachment polymers. From examination of compendia of structural analysis of exopolymers (7), it was clear that uronic acids were the most unique yet universal component found in polymers on the outside of the cytoplasmic membrane in bacteria. A quantitative assay based on the predominance of D-glucuronic, D-galacturonic, D-mannuronic, and L-gulonic acids in these polymers was developed (5). Since the presence of the carboxyl group stabilizes the glycosidic linkage (20), low yields result when the polymers are subjected to acid hydrolysis prior to separation and analysis. Once the uronic acids are released from the polymer, they are subjected to lactonization that is not reproducible. The solution to the quantitative assay problems involved activating the carbonyl group by esterification and then reducing the carbonyl group to an alcohol group with sodium borodeuteride. The uronic acid residues were then reduced to primary alcohols while they were still in place in the polymers but containing the deuterium tracer. The deuterium-containing sugars were readily hydrolyzed and separated by capillary GC after anomeric carbon reduction and peracetylation. The deuterium was detected after electron-impact mass spectrometry and the proportion of uronic acids was estimated. Quantitative recovery of gum arabic polymer D-glucosamine and D-galactosamine confirmed the success of the procedure. Mixtures of polygalactosuronic acid were derivatized, to yield dideuterated galactose, recovered, and mixed with authentic galactose in various proportions. The percentages of added dideuterated galactose correlated with the results of the GC-MS analysis. With this technique, uronic acid-containing polymers were detected in marine pseudomonads, Maldanid worm tubes, ptychodera fecal mounds, biofouled titanium and aluminum, and estuarine sediments (7, 8). It was also possible to

show that cultures of the marine bacterium *Pseudomonas atlantica* formed increased amounts of extracellular polysaccharide glycocalyx with increased proportions of uronic acids as the age of the culture increased (38). The age of monoculture was estimated from the growth curve and the adenylate energy charge. Maximal glycocalyx formation corresponded to conditions of nutritional stress. The faster the rate of exopolymer synthesis, the higher the uronic acid concentration. These data clearly showed that the composition of the exopolymer glycocalyx could change during the growth cycle. During a period of 8 days the total extractable phospholipid content increased 3-fold, the total polysaccharide polymer content increased 24-fold, and the total uronic acid content increased 83-fold, representing an increase in proportions from 8 to 26%. The total content of arabinose and xylose in the polymer remained relatively constant. The galactose/galacturonic acid ratio decreased from 9.3 to 1.8, compared with an increase from 2.4 to 3.4 in the glucose/glucosuronic acid ratio (38). In an unidentified marine bacterium (22), changes were observed in poly-β-hydroxybutyrate (PHB). When these cells were starved for 24 h, total fatty acid levels and the ratio of monounsaturated fatty acids to saturated fatty acids decreased, and the level of short-chain fatty acids increased. Initially, starving cells contained PHB, but after 3 h no PHB was detected. Addition of a phosphorus buffer permitted an initial increase in PHB and then a prolonged delay prior to the disappearance of PHB.

The chemical detection of the glycocalyx by derivatization, hydrolysis with GC-MS, and detection of uronic acid proportions was a long and ardous procedure. It provided compositional data only, with little insight into the detailed structure of the glycocalyx. The role of exopolymer structure in attachment requires more detailed study. The preliminary experiments with *P. atlantica* suggested strongly that the cellular attachment strength to metals in seawater was increased as the proportion of uronic acids was increased. Various components of the glycocalyx polysaccharides have been suggested to be critical in the attachment of the cells to substrata. In the polymer of *P. atlantica*, ~~the~~ uronic acids, ~~the~~ acetylated hydroxyl groups, ~~hydroxyls~~, and ~~the~~ pyruvate acetals all could contribute charged or hydrophobic groups. Attempts to change the adhesive strength of the isolated polymer or the cells by chemical means, such as methylating the polymer uronic acids, did result in a much less adherent polymer, but fragmentation in the polymer, deacetylation, and methylation of the uronic acids resulted under even the gentlest conditions for ester formation. Chemical modifications of polymers appeared to be just too blunt an instrument with which to dissect the structure-adhesion relationships.

GENETIC ANALYSIS

The sharp instrument with which to study the effects of specific changes in the extracellular polymers that result in changes in the specificity and strength of adhesion is at hand: the creation of mutants with modifications in the structure of the exopolymers. A dramatic and uncontestable change related to adhesion was detected in *Vibrio parahaemolyticus*. The irreversible attachment to substrata coincided with the elaboration of thin lateral flagella by the bacteria. These lateral flagella were clearly distinct from the sheathed polar flagellum used for motility. Genetic analysis by ~~Silverman and co-workers~~ Belas et al. (2) has shown that genes for production of lateral flagella are switched on about 30 min after the organism has made contact with a surface. Clearly, a surface-sensing event has taken place (19). By transposon genetic manipulation [mini-Mu(LacZ Tet^r)], Belas et al. (2) were able to isolate peritrichous tetracycline-resistant *V. parahaemolyticus* with defective lateral flagella (nonswarmers). Since *V. parahaemolyticus* is ~~Lac$^-$~~ Lac negative nonswarmers were tested for β-galactosi-

dase activity as a function of induction of lateral flagella. In at least 40% of the isolates, a surface-dependent β-galactosidase activity on agar, nitrocellulose, cellulose acetate, and wetted polyvinylidene fluoride filters was demonstrated. The time course of appearance for surface-dependent β-galactosidase was the same as that observed for production of lateral flagella (2). Silverman and co-workers were able to isolate the genes for bioluminesence from *Vibrio fischeri* (6) and have used them in the same way as the β-galactosidase to detect the activity of the specific operon that contains the genes. Thus, the activity of specific genes involved in adhesion can be monitored in real time as the adhesion process continues. The elegant work has provided the tools required to modify the exopolymer or extracellular organelle involved in irreversible adhesion once the specific mechanism(s) is known.

METHODS OF MEASUREMENT OF ADHESION

The creation of mutants with different adhesive propensities provides the biological material for the analysis of the role of extracellular polymers in adhesion. Clearly, chemical analysis of polymers provides insight into the factors affecting formation; however, new methods, particularly if they can be rapid and nondestructive, provide a means of interpreting the molecular consequences of the mutations involving adhesive properties.

Shear Force

One method of measuring adhesion is to apply a fixed or variable force to adhered cells. Adhesion is then expressed as a percentage of the cells which withstand the applied disruptive force (e.g., gravity flow, gentle washing, sonication, vortexing, and gravity and resultant buoyant density). One of the most accurate methods of measuring adhesive strength is to use the disk shearing device (40,41), in which a disk is spun at a known rate and distance from another disk with adhered cells. Transmitted shear stress is then dependent upon rotation rate, separation distance, fluid viscosity, and radial position. These methods measure the adhesiveness of the fully formed biological adhesion joint. Dynamic assessment of adhesion, however, sets up a fluid shear against which the cell must adhere. The cells are carried along in a cylinder of nutrient liquid between two parallel test surfaces separated by a distance h. Opposing forces (attraction to the test surface and fluid shear) act upon the cells, and there is a mean residence time during which the cells are in contact with the test surfaces. The time required for biological adhesion to occur is then dependent upon the biological adhesion potential of the cells (15). By using the cell adhesion module, the dynamic shear force required for a cell to become attached to a surface can be determined as a function of the radius, r. In the cell adhesion module there are two types of flow rates: (i) the linear (overall) flow rate, which is set to ensure laminar flow (Reynolds number $[R_n] < 2{,}000$) (Fig. 1), and (ii) the decreasing differential flow of nutrient medium between the plates of the cell adhesion module. The differential flow results from the ever-increasing volume (r is continuously increasing) which must be filled (volume $= \pi \times r^2 \times h$) as the front of the flowing medium moves from the center of the test surface to beyond its outer edge, where the flow again becomes linear. The cells which form the inner ring of the biofilm (Fig. 2) are the most strongly adherent cells, since they are attached to the test surface at a point of higher differential flow (higher shear force) than are the cells at the periphery. By measuring the radius formed by the most strongly adherent cells, the maximum dynamic shear force can then be calculated (15). A variety of techniques exist for marking the inner boundary of the biofilm with

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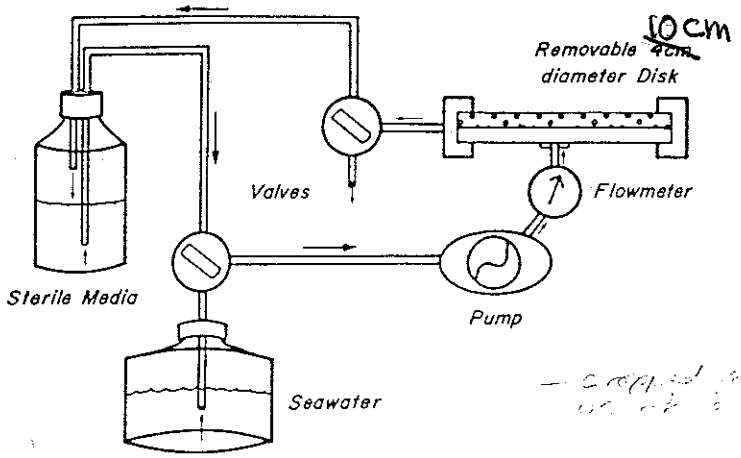

Figure 1. Cell adhesion module, used to monitor microbial attachment (adhesive strength) in a controlled shear gradient. Bacterial monoculture is pumped through the cell in a continuous-flow system as depicted or in a closed batch system (not shown). The test surface here is a glass disk (diameter, 10 cm) and is removable. Laminar flow rates are used (Reynolds number, <2,000), and the shear force, τ is calculated from $\tau = 3Q\mu/\pi rb^2$, where Q is total volumetric flow rate (in cubic centimeters per second), μ is the dynamic viscosity of the medium (in centipoise), r is the radius (in centimeters), and b is the vertical distance between the plates (in centimeters).

attached cells (measurement of diffuse reflectance by Fourier transforming infrared spectroscopy [FT-IR] [28]), biological staining and epifluorescence microscopy, tritium exchange and autoradiography, and destructive extraction followed by chromatography for signature compounds (36, 37, 43).

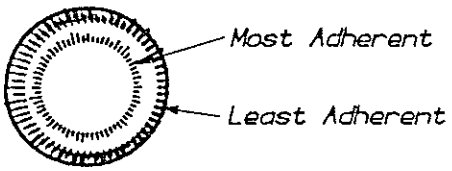

Figure 2. Cell adhesion module disk. The disk from the cell adhesion module (diameter, 10 cm, with attached microbial film and bacteria, is shown. The inner ring contains the most strongly adherent bacteria, since they have become attached in a region of high shear force compared with the least strongly adherent cells (attached under lower shear force). The cells become attached to the removable disk, forming a clear zone around the center inlet; the outer radius of the clear zone is measured and used to calculate the maximum dynamic shear force for the given bacterial strain. The mean least radius (maximum shear force) of attachment is monitored by a variety of methods (see the text).

Nondestructive Methods

AC Impedance

The basic concept of impedance measurement methods lies in the development of an electrical field structure at the electrode interface and a different electrical field structure in the bulk medium. The electrical interface (electrode/bulk medium) forms a compromise structure. To measure microbial growth, the impedance or the capacitance of the bulk medium is measured (32); however, the effects of adhesion require a surface to which the organisms can attach. Therefore, the methodology must allow for removal of the effects of bulk phase changes (e.g., bacterial growth, pH, charged species,

and solvent dipoles) and analyze only the electrode interface changes. This region of the electrode/electrolyte interface is electrically neutral, but has a potential difference across the interface (the electrical double layer) (10). Therefore, the electrobacteriological experimental setup consists of at least two electrodes placed in the growth medium chambers which have been inoculated with the appropriate cultures. At each electrode there is an electrical double layer with its own direct current (DC) boundary potential. When an alternating current (AC) signal is impressed across these electrodes, the DC boundary potential becomes modulated by the AC signal and the resulting impedance can be calculated. When the appropriate experimental protocol (dual-chamber cells separated by a membrane filter and multiple electrodes) (21) is followed, the resultant impedance provides a calculated measure of the events at the test surface interface electrode. Practical applications of AC impedance methods (4) have shown that it is possible to measure microbially facilitated corrosion rates repeatedly in the same biofilm. This allows the detection of the consequences of succession in the biofilms. The measurements have been shown to correlate with the classical potentiometric DC measurements of corrosion rates (5). The DC methods unfortunately impress such large voltages on the surface that the measurements are destructive to the biofilm. AC impedance measurements offer the potential of nondestructive monitoring of biofilm chemistry, particularly when correlated with other nondestructive methods.

FT-IR

FT-IR provides spectral advantages sufficient to allow the examination of biofilms (27, 43). When FT-IR is used with the attenuated total reflectance (ATR) cell, living biofilms can be detected. With ATR, the incident light passes through the crystal a given number of times that are dependent upon the angle at which the crystal face was cut. The attenuated emitted light is then detected by the FT-IR spectrometer. The ATR system involves a crystal of germanium or zinc selenide shaped in such a way that the IR spectrometer sees an evanescent wave extending about 0.5 μm into the test system (Fig. 3). The IR spectra of whatever becomes attached to the crystal surface to the edge of the evanescent wave can be detected continuously and nondestructively. If a cell is created in which seawater flows over the cell (Fig. 3), the formation of biofilms can be detected. The formation of the conditioning film on surfaces exposed to seawater was readily monitored, and the steady accumulation of carbohydrate was monitored as an increase in the ether stretch absorbance (27). Geesey et al. (1) was used FT-IR with ATR to directly demonstrate the corrosion of copper in seawater by the deposition of acidic bacterial polymers on the surface of the copper. They sputter-coated the ATR crystal with a thin film of copper, placed the crystal in a cell, and compared the effects of seawater with those of seawater plus attached bacterial exopolymer. They detected corrosion by measuring the accumulation of copper in the seawater and by directly watching the increase in water absorption at 1,640 cm^{-1} as the copper was removed from the film. Recent experiments (unpublished data) in our laboratory by Nivens have shown that the attachment of *Caulobacter* species to the ATR crystal is correlated with the appearance of bacterial proteins and polysaccharides in the IR spectra. By using a crystal sputter-coated with a thin film of metal such as stainless steel, it will be possible to simultaneously monitor the attachment of bacteria to the coated crystal by FT-IR and AC impedance techniques. The effects of changing the electrical potential of the metals on adhesion chemistry of the bacteria can then be observed.

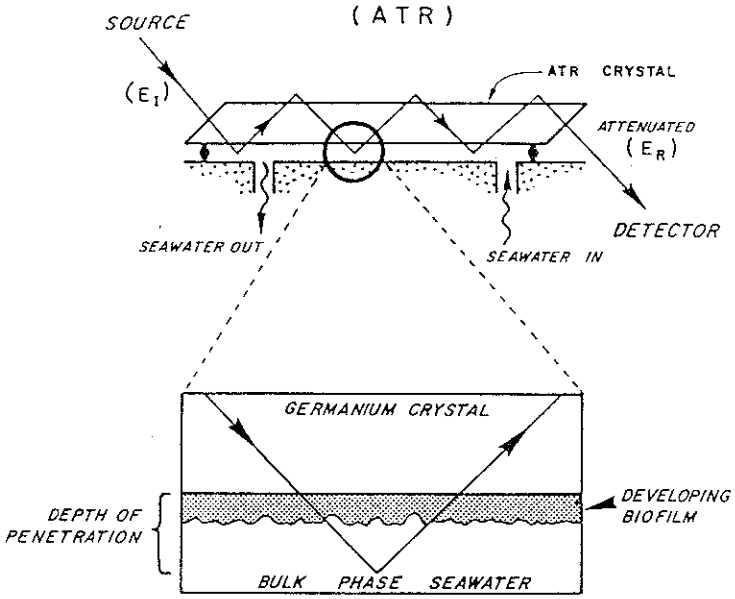

Figure 3. ATR schematic. The incident source of radiant energy impinges upon and is reflected through the ATR crystal. The angle of reflection and the number of times the light beam is reflected before it is detected are dependent upon the angle at which the crystal face was cut. Background spectra (without any attached biofilm) can be subtracted from the spectra taken as the biofilm (and as the bacteria) attach, and wavelengths characteristic for the molecules of interest can be examined.

Surface-Enhanced Raman Spectroscopy

Surface-enhanced Raman spectroscopy is a nondestructive technique that complements FT-IR. In this analysis the molecules of material are activated by exposure to visible light from a laser. The Raman scattering of light from molecules results in a color shift in a portion of the scattered light by conversion of quanta to the vibrational energy of molecules. This is detected in a different region of visible light, with the difference representing the vibrational spectra of the activated molecules. Because the exciting and emitted light are both in the visible portion of the electromagnetic spectrum, the problems with solvents that are associated with the FT-IR are obviated. The absorption pattern of water, carbon dioxide, glass, etc., is not a problem in Raman spectroscopy, since any optically clear component is clear to the Raman spectrometer. In the past, the problem with Raman spectroscopy has been lack of sensitivity. Generally, Raman scattering is ~~1,000-fold~~ much less ~~sensitive~~ intense than ~~the standard response~~ Rayleigh scattering. Recently it has proved possible to enhance the intensity of Raman scattered light by a factor of 10^6 when the molecules of interest form a monolayer on a metallic surface. The enhancement of polarizability of molecules in the electron plasmon of silver has proved the most effective method of increasing the sensitivity of Raman spectroscopy to date (9). These developments mean that an apparatus like that illustrated in Fig. 4 could be developed. If the fiber-optic probe is used, it should be possible to detect and identify molecules attaching to the silver granules in specific parts of the biofilm.

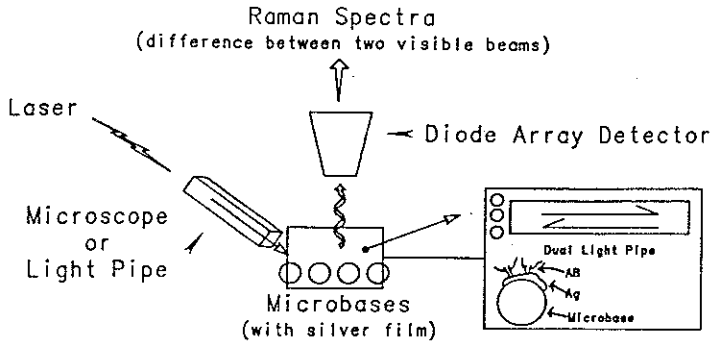

Figure 4. Surface-enhanced Raman spectroscopy schematic. The incident light from the laser can be focused through a microscope or fiber-optic tube on the microbases on which the silver surface is deposited. The plasmon of the silver on the microbases enhances the Raman scattered light from the molecules deposited on the microbases, and the scattered light is detected by a diode array detector. The surface-enhanced Raman spectroscopy system can be made specific (inset) by attaching antibodies or enzymes to the microbases and analyzing the changes in conformation when the molecule reacts with its antigen or substrate.

Ultrasensitive Destructive Measurement Techniques

Examination of biofilm development in monoculture or use of microbial consortia very often is limited by the sensitivity of the instruments used. To enhance the sensitivity and accuracy of these measurements (femto- and attomolar ranges), high-purity gases and solvents, efficient extractions, and ultrasensitive instruments are used. The methods developed for the extraction and concentration of cellular components, particularly of the membrane phospholipids (42), will be used prior to purification by high-pressure liquid chromatography and SFC. These membrane - lipopolysaccharide - exopolymer components are further separated by SFC or capillary GC for analysis by MS. If electron-withdrawing derivatives and soft chemical-ionization MS with detection of negative ions (29) are used, the sensitivity can be increased significantly. Direct analysis of biofilms or isolated cellular components has recently been shown by using fast-atom bombardment MS (see chapter 39 of this volume). ~~This can increase the utility significantly~~ and may be used to show whether specific microorganisms are located in specific parts of a biofilm. All of these analytical methods provide quantitative structural data for biofilm adhesive joints that are finished.

CONCLUSIONS

Applications of current techniques, when combined with mutants selected for their differences in adhesive properties, can yield considerable insight into the factors that control the elaboration of exopolymers. The questions of the mechanisms by which bacteria adhere to surfaces, the role(s) of extracellular polymer in attachment and adhesion, the strain or species divergent evolutionary mechanism(s) of attachment, and the signaling events which result in detectable physiological changes all deal with small numbers of cells (less than 10^4 cells per ml), and the limits of instrumental detection are easily reached. In some cases, observation and analysis of the attachment of one bacterium are required. The development of new nondestructive methodologies with several-fold increases in sensitivity will herald the

next stage of understanding of this essential feature of the microbial mat.

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LITERATURE CITED

1. Baier, R. E. 1984. Initial events in microbial film formation, p. 57–62. *In* J. D. Costlow, and R. C. Tipper (ed.), *Marine Biodeterioration: an Interdisciplinary Study*. Naval Institute Press, Annapolis, Md.
2. Belas, R., L. McCarter, M. Simon, and M. Silverman. 1983. Genetic basis of bacterial surface recognition, p. 12–13. *In Proceedings of the Conference of Microbial Adhesion and Corrosion in the marine Environment*. The Agouron Institute, La Jolla, Calif.
3. Costerton, J. W., T. J. Marrie, and K.-J. Cheng. 1985. Phenomena of bacterial adhesion, p. 3–43. *In* D. C. Savage and M. Fletcher (ed.), *Bacterial Adhesion*. Plenum Publishing Corp., New York.
4. Dowling, N. J. E., J. Guezennec, and D. C. White. 1987. Facilitation of corrosion of stainless steel exposed to aerobic seawater by microbial biofilms containing both facultative and absolute anaerobes, p. 27–38. *In* E. C. Hill, J. L. Sherman, and R. J. Watkinson (ed.), *Microbial Problems in the Offshore Oil Industry*. John Wiley & Sons, Ltd., Chichester, England.
5. Dudman, W. F. 1977. The role of surface polysaccharides in natural environments, p. 357–414. *In* I. Sutherland (ed.), *Surface Carbohydrates of the Prokaryotic Cell*. Academic Press, Inc., New York.
6. Engebrecht, J., K. Nealson, and M. Silverman. 1983. Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell* 32:773–781.
7. Fazio, S. A., D. J. Uhlinger, J. H. Parker, and D. C. White. 1982. Estimations of uronic acids as quantitative measures of extracellular and cell wall polysaccharide polymers from environmental samples. *Appl. Environ. Microbiol.* 43:1151–1159.
8. Fazio, S. D., W. R. Mayberry, and D. C. White. 1979. Muramic acid assay in sediments. *Appl. Environ. Microbiol.* 38:349–350.
9. Ferrell, T. L., T. A. Callcott, and R. J. Warmack. 1985. Plasmons and surfaces. *Am. Sci.* 73:344–353.
10. Firstenberg-Eden, R., and G. Eden. 1984. *Impedance Microbiology*, p. 7–34, 41–70, and 73–134. ~~Research Studies Press, Letchworth, England.~~ John Wiley & Sons, Inc., New York.
11. Fletcher, M. 1976. The effects of proteins of bacterial attachment to polystyrene. *J. Gen. Microbiol.* 94:400–404.
12. Fletcher, M. 1986. Effect of solid surfaces on the activity of attached bacteria, p. 339–400. *In* D. C. Savage and M. Fletcher (ed.), *Bacterial Adhesion*. Plenum Publishing Corp., New York.
13. Fletcher, M., and G. D. Floodgate. 1976. The adhesion of bacteria to solid surfaces, p. 101–107. *In* R. Fuller and D. W. Lovelock (ed.), *Microbial Ultrastructure*. Academic Press, Inc. (London), Ltd., London.
14. Fletcher, M., and K. C. Marshall. 1982. Bubble contact angle method for evaluating substratum interfacial characteristics and its relevance to bacterial attachment. *Appl. Environ. Microbiol.* 44:184–192.
15. Fowler, H. W., and A. J. McKay. 1980. The measurement of microbial adhesion, p. 48–66. *In* R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent (ed.), *Microbial Adhesion to Surfaces*. Ellis Horwood Ltd., Chichester, England.
16. Geesey, G. G. 1982. Microbial exopolymers: ecological and economic considerations. *ASM News* 48: 9–14.
17. Geesey, G. G., M. W. Mittleman, T. Iwaoka, and P. R. Griffiths. 1986. Role of bacterial exopolymers in their deterioration of metallic copper surfaces. *Mater. Perf.* 25:37–40.
18. Hedrick, D. B., and D. C. White. 1986. Rapid extraction and fractionation of particulate BW agents for detection of signature biomarkers by mass spectrometry, p. 1–134. *In Proceedings of the U.S. Army Chemical Defense Research and Engineering Center Conference. Edgewood Area.* ••••••••.
19. Lewin, R. 1984. Microbial adhesion is a sticky problem. *Science* 224:375–377.
20. Lindberg, B., J. Longren, and S. Svensson. 1975.

In M. D. Rouser (ed.) Proceedings of the ... Edgewood Area, U.S. Army Armaments, Munitions, and Chemical Command, Aberdeen Proving Ground. Code CRDC-SP-87008

Specific degradation of polysaccharides. *Adv. Carbohydr. Chem. Biochem.* 31:185–239.

21. Little, B. J., P. Wagner, S. M. Gerchakov, M. Walch, and R. Mitchell. 1986. The involvement of a thermophilic bacterium in corrosion processes. *Corrosion* 42:533–536.
22. Malmcrona-Friberg, K., A. Tunlid, P. Marden, S. Kjelleberg, and G. Odham. 1986. Chemical changes in cell envelope and poly-beta-hydroxybutyrate during short term starvation of a marine bacterial isolate. *Arch. Microbiol.* 144:340–345.
23. Marshall, K. C., R. Stout, and R. Mitchell. 1971. Selective sorption of bacteria from seawater. *Can. J. Microbiol.* 17:1413–1416.
24. McEldowney, S., and M. Fletcher. 1986. Effect of growth conditions and surface characteristics of aquatic bacteria on their attachment to solid surfaces. *J. Gen. Microbiol.* 132:513–523.
25. Mirelman, D., and I. Ofek. 1986. Introduction to microbial lectins and agglutinins, p. 1–19. *In* D. Mirelman (ed.), *Microbial Lectins and Agglutinins.* John Wiley & Sons, Inc., New York.
26. Mitchell, R., and D. Kirchman. 1984. The microbial ecology of marine surfaces, p. 49–56. *In* J. Costlow and R. C. Tipper (ed.), *Marine Biodeterioration: an Interdisciplinary Study.* Naval Institute Press, Annapolis, Md.
27. Nichols, P. D., J. M. Henson, J. B. Guckert, D. E. Nivens, and D. C. White. 1985. Fourier transform-infrared spectroscopic methods for microbial ecology: analysis of bacteria, bacteria-polymer mixtures and biofilms. *J. Microbiol. Methods* 4:79–94.
28. Nivens, D. E., P. D. Nichols, J. M. Henson, G. G. Geesey, and D. C. White. 1986. Reversible acceleration of corrosion of stainless steel exposed to seawater induced by the extracellular secretions of the marine vibrio *V. natriegens*. *Corrosion* 41:201–210.
29. Odham, G., A. Tunlid, G. Westerdahl, L. Larsson, J. B. Guckert, and D. C. White. 1985. Determination of microbial fatty acid profiles at femtomolar levels in human urine and the initial marine microfouling community by capillary gas chromatography-chemical ionization mass spectrometry with negative ion detection. *J. Microbiol. Methods* 3:331–344.
30. Pethica, B. A. 1980. Microbial and cell adhesion, p. 19–45. *In* R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent (ed.), *Microbial Adhesion to Surfaces.* Ellis Horwood Ltd., Chichester, England.
31. Platt, R. M., G. G. Geesey, J. D. Davis, and D. C. White. 1986. Isolation and partial chemical analysis of firmly bound exopolysaccharide from adherent cells of a freshwater sediment bacterium. *Can. J. Microbiol.* 31:675–680.
32. Pringle, J. H., M. Fletcher, and D. C. Ellwood. 1983. Selection of attachment mutants during the continuous culture of *Pseudomonas fluorescens* and the relationship between attachment ability and surface composition. *J. Gen. Microbiol.* 129:2557–2569.
33. Radford, T., and D. C. DeJongh. 1972. Carbohydrates, p. 313–349. *In* G. R. Waller (ed.), *Biochemical Applications of Mass Spectrometry.* John Wiley & Sons, Inc., New York.
34. Rosenberg, M., and S. Kjelleberg. 1986. Hydrophobic interactions: role in bacterial adhesion. *Adv. Microb. Ecol.* 9:353–393.
35. Schwartz, H. E., P. J. Barthel, S. E. Moring, and H. H. Lauer. 1987. Packed vs. capillary column for supercritical fluid chromatography. *LC-GC.* 5:490–498.
36. Tunlid, A., B. H. Baird, M. B. Trexler, S. Olsson, R. H. Findlay, G. Odham, and D. C. White. 1985. Determination of phospholipid ester-linked fatty acids and poly beta hydroxybutyrate for the estimation of bacterial biomass and activity in the rhizosphere of the rape plant *Brassica napus* (L.). *Can. J. Microbiol.* 31:1113–1119.
37. Tunlid, A., G. Odham, R. H. Findlay, and D. C. White. 1985. Precision and sensitivity in the measurement of ^{15}N enrichment in D-alanine from bacterial cell walls using positive/negative ion mass spectrometry. *J. Microbiol. Methods* 3:237–245.
38. Uhlinger, D. J., and D. C. White. 1983. Relationship between physiological status and formation of extracellular polysaccharide glycocalyx in *Pseudomonas atlantica*. *Appl. Environ. Microbiol.* 45:64–70.
39. Ward, J. B., and R. C. W. Berkeley. 1980. The microbial cell surface and adhesion, p. 48–66. *In* R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent (ed.), *Microbial Adhesion to Surfaces.* Ellis Horwood Ltd., Chichester, England.
40. Weiss, L. 1961. The measurement of cell adhesion. *Exp. Cell Res. Suppl.* 8:141–153.
41. Weiss, L. 1961. Studies on cellular adhesion in tissue culture. IV. The alteration of substrata by cell surfaces. *Exp. Cell Res.* 25:504–517.
42. White, D. C. 1986. Environmental effects testing with quantitative microbial analysis: chemical signatures correlated with *in situ* biofilm analysis by FT/IR. *Toxicity Assessment* 1:315–338.
43. White, D. C., D. E. Nivens, P. D. Nichols, A. T. Mikell, B. D. Kerger, J. M. Henson, G. G. Geesey, and C. K. Clarke. 1986. Role of aerobic bacteria and their extracellular polymers in the facilitation of corrosion: use of Fourier transforming infrared spectroscopy and "signature" phospholipid fatty acid analysis, p. 233–243. *In* S. C. Dexter (ed.), *Biologically Induced Corrosion.* National Association of Corrosion Engineers reference book 8, National Association of Corrosion Engineers, Houston, Tex.