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Theoretical and Practical Significance of Bacteria at Interfaces

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Solid-liquid, gas-liquid, and liquid-liquid interfaces represent sites of potential nutrient accumulation in nutrient-deficient habitats and, consequently, they exert a marked influence on microbial distribution, metabolism, growth, and succession in such habitats. Aspects of the behavior of different bacteria at surfaces can be anticipated following investigations on the physiological and colloidal properties of the bacteria, the nature and extent of the interfaces involved, and the degree of modification to the interfaces resulting from adsorption to the interfaces of conditioning films. With this knowledge, a greater insight is possible for the definition, understanding, and solution of many practical microbiological problems.

INTRODUCTION

The nutrient status of most natural habitats is too low to support active growth of microorganisms, and we are becoming increasingly aware of the marked effects of interfaces on the ability of microorganisms to exploit the nutrients available in such habitats. Nutrients are concd at a variety of solid-liquid, gas-liquid, and liquid-liquid interfaces, and the degree and type of microbial responses are related to the qualitative and quantitative nature of the available interfaces in any particular habitat. Bacteria possess a net negative surface charge and behave as colloidal particles in that they normally form stable suspensions in the aqueous phase. One can obtain an insight into the behavior of different bacteria at interfaces by a careful consideration of both the colloidal and biological properties of various species in different habitats (Marshall 1976). The questions raised by the above generalizations are: why do nutrients accumulate at interfaces; what responses are induced in the bacteria; do all bacteria respond in the same manner; what is the nature of adhesion of bacteria to solid surfaces; and are such bacterial-interface interactions of any significance?

DISCUSSION

Nutrient Accumulation at Interfaces

It is well established that the physiochemical properties of interfaces differ from those existing within either phase in a two-phase system. These unique interfacial forces have a significant effect on the distribution of ions, macromolecules, and colloids in the vicinity of the interfaces. We know that most interfaces assume a net negative charge and, consequently, attract cations and a variety of macromolecules. For example, some proteins are denatured and spread to form monolayers at interfaces.

Materials accumulate at air-water interfaces to form surface microlayers (Parker and Barsom 1970), and may originate from rain, atmospheric fallout, runoff, or the subsurface water. Bubble formation provides an effective means for adsorption, concn, and transport of nutrient ions and macromolecules to the surface. This phenomenon, termed the adsubble (adsorptive bubble separation) process by Lemlich (1972), results in a selective concn at the surface of bacteria derived from the water column (Carlucci and Williams 1965). This selectivity in adsorption and transport may reflect differences in degrees of hydrophilicity and hydrophobicity of the mixed bacterial population in the water column (Marshall 1976).

Immersion of solids into natural aqueous systems results in a spontaneous adsorption of macromolecular conditioning films to the solid surface. Such films alter the surface free energy, as determined by critical surface tension measurements (Baier et al. 1968), and markedly influence the behavior of bacteria near the solid-liquid interface. Amphipathic polymers adsorb to different surfaces at the same rate but in different configurations, and this influences the nature of bacterial adhesion at such conditioned surfaces (Baier 1978).

Attraction of Bacteria to Interfaces

Motile bacteria exhibit a chemotactic response along the nutrient gradient established adjacent to interfaces in oligotrophic conditions (Young and Mitchell 1973). Other mechanisms of attraction must exist, since many of the organisms accumulating at such interfaces are nonmotile. Cell-surface hydrophobicity is a major factor influencing the behavior of bacteria at interfaces. Mudd and Mudd (1924) demonstrated that, if γ_{ow} is the surface tension at an oil-water interface, γ_{bw} at the bacterial-water interface, and γ_{bo} at the bacterial-oil interface, then a bacterium moves into the oil phase where $\gamma_{bw} > \gamma_{bo} + \gamma_{ow}$ and it moves into the water phase when $\gamma_{bo} > \gamma_{bw} + \gamma_{ow}$. We have demonstrated that the perpendicular orientation of *Flexibacter* CW7 at air-water, oil-water, and solid-water interfaces (Fig. 1) and the process of rosette formation within the aqueous phase (Fig. 2) result from a tendency for the more hydrophobic, pointed end of the cells to be rejected from the aqueous phase (Marshall and Cruickshank 1973).

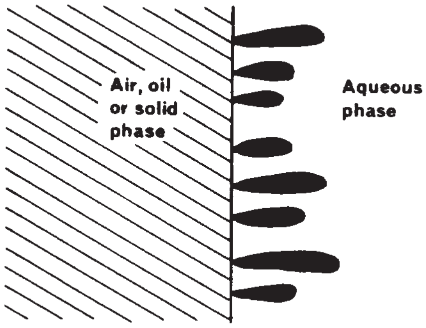


FIG. 1 Perpendicular orientation of *Flexibacter* CW7 at air-water, oil-water, and solid-water interfaces. Note that the pointed, hydrophobic pole of the cell faces away from the aqueous phase (adapted from Marshall and Cruickshank 1973).

FIG. 2 Rosette formation in the aqueous phase by *Flexibacter* CW7, with the pointed, hydrophobic poles of each cell facing inward (adapted from Marshall and Cruickshank 1973).

Electrostatic attraction of negatively charged bacteria to an interface would occur only where the interface assumed a positive charge. Attraction of bacteria to negatively charged interfaces depends upon interacting electrical double layer repulsion and van der Waals attraction energies, the resultant energy being dependent upon the electrolyte valency and concn. We have shown that numbers of a nonmotile *Achromobacter* sp. attracted to a glass surface decreased with decreasing electrolyte concn until a point was reached where all bacteria were repelled from the surface (Marshall et al. 1971). The critical concn at which repulsion occurred were 5×10^{-4}M for NaCl and 5×10^{-5}M for $MgSO_4$. The bacteria attracted to the surface were not firmly attached, but held at a small, finite distance from the surface. We termed this process reversible sorption. It is of interest to note that the kinetic energy of a motile marine pseudomonad (5.45×10^{-18} ergs) was insufficient to overcome the repulsion energy barrier (50×10^{-13} ergs) holding the bacterium from the surface.

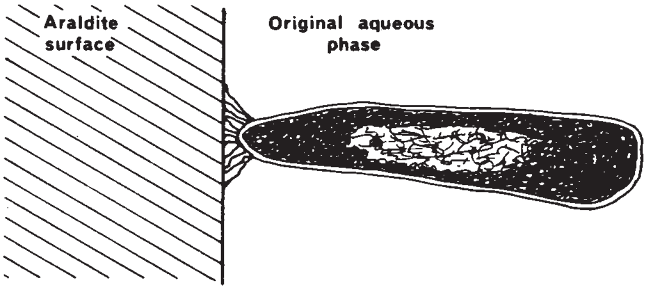
Adhesion of Bacteria to Solid Surfaces

We now know that true adhesion of bacteria to surfaces may be either temporary or permanent, and that permanent adhesion may be of a nonspecific or a specific nature.

Permanent Adhesion

Nonspecific adhesion. This process occurs with those bacteria capable of permanent adhesion to a variety of solid surfaces, and is the usual form of adhesion observed in natural habitats. The repulsion-energy barrier between the bacterial and solid surfaces is overcome by a combination of the biological and physical properties of bacteria. Some, but not all, bacteria respond to the enriched nutrient status at interfaces by producing extracellular acidic polysaccharides or glycoproteins, which anchor the cells to surfaces by the process of polymer bridging (Fig. 3). The fibrillar nature of the polymer in the perpendicularly attached *Flexibacter* CW7 was emphasized in those sections we cut obliquely to the original solid-water interface (Fig. 4).

There is a growing awareness that the extent and strength of nonspecific adhesion of bacteria to surfaces are dependent upon the bacterial species and the initial surface properties of the solids involved. Dexter et al. (1975) and Baier (1978) reported that surfaces with



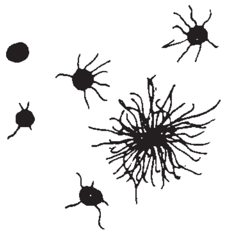


Fig. 3 Section of *Flexibacter* CW7 permanently adhering to an araldite surface in a perpendicular manner. Note the polymer bridging between the cell and solid surface (adapted from Marshall and Cruickshank 1973).

Fig. 4 An oblique section across the original araldite-water interface to demonstrate the polymer fibrils attaching *Flexibacter* CW7 to the araldite surface (adapted from Marshall 1976).

initial critical surface tension (γ_c) values of about 20 to 30 dynes cm^{-1} exhibit minimum bacterial adhesion, whereas surfaces with γ_c values above or below these limits supported extensive colonization. On the other hand, Fletcher and Loeb (1976) found greater attachment on surfaces with low γ_c values and a low negative charge than on surfaces with high γ_c values. They used materials with γ_c values of 19 and greater than 31 dynes cm^{-1}, but did not test materials within the apparently critical range given above. In addition, different organisms and other conditions were employed by the various authors. Baier (1978) suggests that the strength of adhesion to surfaces within the range of γ_c values from 20 to 30 dynes cm^{-1} is less than to other surfaces, and may be a reflection of the degree of apparent denaturation of the macromolecules masking the original immersed surfaces.

Specific adhesion. The attachment of particular bacteria to specific surfaces must involve interactions between complementary molecular configurations on the bacterial and solid surfaces. In the enteropathogenic *Escherichia coli* K88, for instance, the external bacterial K88 antigen reacts specifically with terminal β-D-galactosyl residues in a mucinous glycoprotein from the small bowel of piglets (Gibbons et al. 1975). Specific attachment in aquatic habitats has not been examined in detail, although the association of certain bacteria with cyanobacterial heterocysts (Paerl 1976) and luminescent bacteria with fish (Reichelt et al. 1977) may be of a specific nature.

Temporary Adhesion

Gliding bacteria attach to and glide across solid surfaces, and are incapable of gliding in the aqueous phase. We have shown (Humphrey et al., unpubl. data) that the extracellular slime produced by a flexibacter is suitable as a temporary (Stefan) adhesive since it exhibits viscous properties characteristic of a linear polymer. This slime increases the adhesiveness (the force preventing separation) but allows translational motion across the surface (a relatively low horizontal drag).

Practical Significance of Interfacial Effects

A realization of the significance of interfaces in the ecology of microorganisms provides a sound basis for the study of many microbial processes in natural situations. Below are examples of some problems we have considered in relation to interfacial phenomena.

Marine fouling. It is established that the colonization by bacteria of surfaces immersed in seawater is a prelude to macrofouling by barnacles and algae. Primary microbial films themselves constitute a significant form of fouling, particularly on the hulls of high-speed vessels where the maintenance of streamlined flow over surfaces is important. Bacteria are small enough to function within stationary boundary layers, and are not affected by the shear forces existing beyond those layers at high speed.

Attempts to overcome microbial fouling in the past have been based on empirical procedures, but we can look forward to a more reasoned approach to the problem in the future, based on knowledge of bacterial behavior at interfaces. We have shown (Marshall 1973) that inhibition of protein synthesis by chloramphenicol prevents most marine bacteria from utilizing the nutrients concd at solid-liquid interfaces for the synthesis of bridging polymers for attachment to the solid surface (Fig. 5). Such results provide a rationale for the incorporation of suitable inhibitors of microbial growth into anti-fouling paints. Some success has been reported in the modification of surface properties to reduce the chemotactic response of marine bacteria to the concn gradient established at solid-liquid interfaces (Chet et al. 1975). An approach to minimizing microbial fouling by nontoxic methods was proposed by Baier (1978), and is based on the control of substrata surface properties to ensure that the initial critical surface tension values are between 20 and 30 dynes cm^{-1}.

Bioconcentration of heavy metals. In aquatic systems heavy metals usually are associated with suspended particulates. Since bacteria readily adhere to particulates, we have examined the tolerance to and accumulation of zinc and cadmium by bacteria isolated from particulates. Most bacteria isolated from particulates contained in water sampled from near a sewage outfall were tolerant of moderate levels of zinc and cadmium, and accumulated high levels of these metals (Hughes and Marshall, unpubl. data). These bacteria can accumulate the heavy metals concentrating at solid-liquid interfaces and, by being ingested by organisms at higher trophic levels, can be regarded as the starting point in the chain of bioconcentration.

Oil biodegradation. Although nutrients accumulate at oil-water interfaces, a significant factor limiting oil degradation by microorganisms is the interfacial area available for microbial colonization. Many microorganisms produce emulsifying agents which assist in increasing the available interfacial area, but commercial detergents are employed in major oil spills to hasten the dispersion of the oil slicks. We know that many commercial detergents are toxic to marine plants and animals, but little is known of their effects on microorganisms apart from the known toxic effects of many cationic and anionic detergents. Recently, we have examined the effect of detergent hydrophile-lipophile balance (HLB values) of an homologous series of nonionic detergents on an *Acinetobacter* sp. (Lupton and Marshall, in press). Both bacterial growth and efficiency of oil degradation increased with decreasing HLB value, the optimum HLB value being about 10 where an effective oil-in-water emulsion was formed. At lower HLB values, water-in-oil emulsions formed and both growth and oil degradation rates declined (Fig. 6).

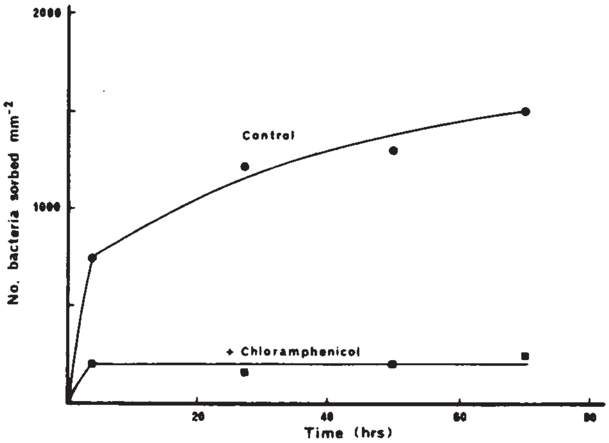


FIG. 5 Effect of chloramphenicol on the adhesion to glass surfaces of bacteria from a natural seawater sample (adapted from Marshall 1973).

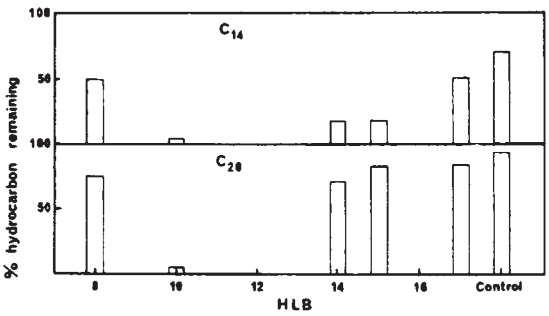
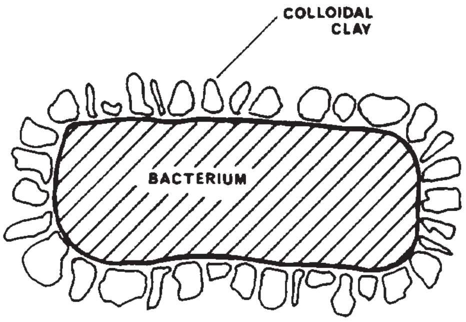


FIG. 6 Effect of detergent HLB on the decomposition of C_{14} and C_{20} hydrocarbons (adapted from Lupton and Marshall, in press).

Estuarine sewage pollution. Numbers of coliform indicator bacteria in the aqueous phase of sewage-polluted estuaries fall rapidly where the salinity exceeds a level given by a specific conductivity of 2.5 mmhos cm^{-1}. We have demonstrated that the coliforms accumulate in sediments, and are protected from natural parasites and predators by sediment colloids (Roper and Marshall 1974 and in press). Even at very low concn of certain colloids (e.g. 100 $\mu g\ ml^{-1}$ of montmorillonite) particles smaller than the bacteria adsorb to the bacterial surface (Fig. 7) and form a protective envelope around the cell (Marshall 1976). Montmorillonite at an effective spherical diameter below 0.6 μ protects *Escherichia coli* from phage attack, whereas clay particles greater than 0.6 μ diameter did not protect the bacteria (Roper and Marshall 1978). Colloidal clay provides some protection from bdellovibrio attack, but not from predation by myxobacters and amoebae. Adsorption of *E. coli* and predators to larger clay particulates results in a physical separation between predator and host (Roper and Marshall in press) and provides a degree of protection to *E. coli* (Fig. 8).



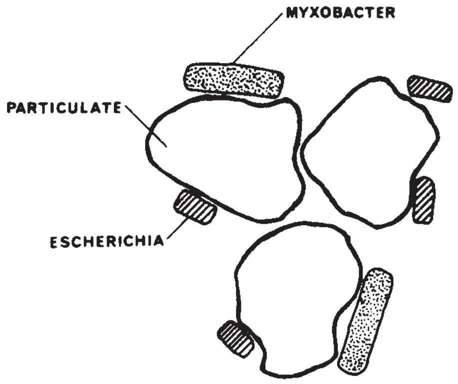


FIG. 7 Diagrammatic representation of colloidal clay particles adsorbed to a bacterial cell.

FIG. 8 Diagrammatic representation of the physical separation between a lytic myxobacter and its host, *Escherichia*, resulting from adsorption of the bacteria to particulate clays.

CONCLUSIONS

We now have a greater understanding of bacterial responses to the unique physiochemical conditions existing at interfaces. By appreciating the modes of attraction of bacteria to interfaces, the mechanisms of attachment, the selectivity of the processes, and the effects of solid surface properties, we have at our disposal invaluable information for application to understand and overcome many practical microbiological problems.

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