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The Dynamics of Biofilms

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ABSTRACT

The colonization of tissue and other surfaces by microbial cells results in the formation of a biofilm. The biofilm mode of existence results from selective pressures in the environment. Biofilms afford microbes greater access to nutrients, protection from antimicrobial agents and provide a buffer to changing conditions in the environment. Micro-organisms that colonize a surface often compete with each other for resources but eventually form consorts which promote their survival. The population structure that develops within a biofilm on living tissue frequently reflects the health of the host organism and its surrounding environment. Displacement of a consort that is beneficial to the host by one that exhibits debilitating characteristics is a common feature of disease. A better understanding of the environmental factors that control microbial activity and population structure within biofilms should promote the development of novel approaches to control the undesirable effects of microbial colonization of surfaces relevant to medical, industrial and environmental processes.

INTRODUCTION

Substantial understanding of the biochemistry and molecular biology of living cells has been achieved through the use of homogeneous

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suspensions of bacterial monocultures. Nevertheless, this approach has fostered the view that micro-organisms are little more than self-replicating sacks filled with molecules of DNA, RNA, protein and lipid. It is not surprising, therefore that suspended monoculture populations of micro-organisms have provided biologists with limited understanding of the diversity of cell–cell interactions that exist in nature or the intimate associations that develop between a cell and its natural environment outside the culture flask.

Like the biochemists' and molecular biologists' disinterest in vectoral metabolism and the organizational and structural importance of membrane-associated reactions proposed in Peter Mitchell's chemiosmotic theory in the early 1960s, many microbiologists today overlook the relevance of these same features when characterizing phenomena involving mixed microbial populations. Metabolically-dependent microbial consortia have evolved in a number of diverse environments. These consortia develop within biofilms on virtually any surface that offers sites for bacterial attachment. The biofilm mode of existence in the microbial world displays chemical gradients, organization and structure that is essential to the existence of microbial populations in most natural environments. This lifestyle promotes efficient processing of energy trapped within a diverse array of chemicals that enter the microbe's environment. In the absence of the chemical gradients and structured microcolonies that develop within biofilms immobilized on surfaces, metabolic capacity and efficiency is severely compromised. These same structural features also offer a degree of protection from the increasing number and abundance of toxic chemicals that have been introduced either intentionally or accidentally to their habitats in recent times.

Previous reviews have demonstrated how bacteria in biofilms are structurally and functionally different from their free-living, planktonic counterparts that commonly exist as homogeneously-dispersed cell populations (Costerton *et al.*, 1987). Lappin-Scott and Costerton (1989) have recently reviewed the microbial associations that exist in the oral cavity of man, the rumen, and on corroded metal piping in industrial water systems. They described how specific surface-associated phenomena are dependent upon the activities of specific physiological types of micro-organism. Geesey and White (1990) have reviewed the methods used to evaluate growth and activities of biofilm populations that develop at the solid–liquid interface.

This paper describes how the structure and organization of microbial populations in surface-associated biofilms develop in response to external stimuli and how subsequent changes in the external environment as well as within the biofilm effect further change in biofilm activity and

population structure, producing a truly dynamic biological system: one that exhibits exquisite adaptability to a constantly fluctuating environment. By understanding the dynamics of biofilm processes, it should be possible to achieve greater success in controlling a wide variety of destructive as well as beneficial microbially-mediated activities in our environment.

WHY A BIOFILM?

Nutritional considerations

A survey of the literature suggests that micro-organisms establish a sessile existence for a variety of reasons. Zobell (1943) first proposed that attachment of marine bacteria to the walls of sampling bottles containing seawater was a response to the enrichment of nutrients at the interface. This idea was further supported by studies showing the capacity of surface-associated marine bacteria to metabolize stearic acid adsorbed to a surface (Kefford *et al.*, 1982; Hermansson & Marshall, 1985). Samuelsson and Kirchman (1990) showed that growth of bacteria on surfaces was related to the amount of degradable protein adsorbed on the surface. Kjelleberg *et al.* (1982) have presented evidence that marine bacteria undergo a variety of physiological changes upon attachment to a surface.

Recently, however, van Loosdrecht *et al.* (1990) questioned this strategy based on physical considerations. They concluded that less than 0·1% of the bacterial cell surface is in contact with the surface and that it is not the higher concentration of adsorbed nutrients that is stimulatory to bacterial growth but rather the faster mass transfer due to a shorter diffusion distance. They concluded from experiments that there was no enhancement of bacterial growth at surfaces at low bulk phase substrate concentrations. They also concluded that the influence of a solid surface on microbiological activity is an indirect effect because the solid influences the medium rather than the bacterium and that there is no conclusive evidence that adhesion directly influences bacterial metabolism, in the sense that bacteria undergo a structural change due to adhesion. Clearly, there is some dispute over the role that nutritional pressures exert on surface attachment by bacterial cells. It is likely that resolution of these issues will require the development of new methods to monitor the expression of specific genes within individual cells as they approach and attach to a surface. To this end, Dagostino *et al.* (1991) have detected changes in gene expression in a marine bacterium upon

attachment to a surface using LacZ fusions. However,the functions of those genes that were 'switched on' as a result of the attachment event have yet to be identified.

Protection against antimicrobial agents

A widely observed response of microbial populations to antimicrobial agents is the establishment of a protective biofilm (Anwar *et al.*, 1989, 1990). Bacteria attached to granular activated carbon (GAC) particles were found to be much more resistant than unattached bacteria to inactivation by chlorine and chloramines (LeChevallier *et al.*, 1984, 1988*a*; Stewart *et al.*, 1990). Mueller *et al.* (1992) demonstrated that cells of *Pseudomonas aeruginosa* adhering to copper surfaces do not replicate due to the toxicity of the metal but replication of cells overlying an initial cell layer was observed with increased exposure time to cell-containing bulk water. Although exopolymers synthesized during biofilm growth are believed to create a barrier to antagonistic agents in the external environment, LeChevallier *et al.* (1984, 1988*b*) found that GAC-attached, polymer-producing bacteria were comparable to non-polymer producing organisms in their resistance to free chlorine or monochloramine.

Bacterial populations that exist as biofilms are also more resistant than free-living, suspended populations of bacteria to antibiotics. Whereas, free-living cells of *Ps aeruginosa* were rendered non-viable after 2 h exposure to 200 μg pipericillin plus 10 μg tobramycin per ml, the same bacteria when grown as a biofilm in a chemostat at a slow growth rate and under iron limitation to the point of senescence retained 0·02% viability when challenged with 200 μg pipericillin plus 100 μg tobramycin per ml (Anwar & Costerton, 1990). Young biofilms were more susceptible than old biofilms to the above combination of antibiotics but unlike the free-living population, were resistant to the individual antibiotics alone. Consequently, minimum inhibitory concentrations of antibiotics and other antimicrobial agents should be determined with biofilm populations of bacteria since this is the mode of growth outside the culture flask where control is of importance. As this topic is discussed more thoroughly in other papers in this issue, it will not be considered any further here. It should be noted, however, that our understanding of the response of pathogens and other unwanted species to antimicrobial agents will require a greater knowledge of biofilm structure before effective and consistent control is achieved.

Formation of cooperative consorts

Biofilms promote interactions between cells of the same and different species that would otherwise be difficult to establish in environments where hydrodynamic forces dominate movement of individual cells. One of the best studied examples is the cooperation of microbial species during cellulose degradation. Microbes within the consortium depend on each other for nutrients, removal of toxic metabolites, and pH and Eh balance. The viscous exopolymers excreted by *Clostridium saccharolyticum* and *Bacteroides celluosolvens* ensure that sugars and growth factors released from the cellulolytic microbe are efficiently delivered to the saccharolytic microbe (Murray, 1986). The exopolymers also anchor the bacteria to the cellulose substrate for efficient enzymatic digestion of the polysaccharide molecules. From the information that is presently available, it appears likely that functional consorts are a common phenomenon in mixed culture biofilms.

Biofilms act as environmentally important reservoirs of microbial diversity. It has been suggested that biofilms (a type of microbial aggregate) contain populations of quiescent cells in their interior that do not become moribund but rather are preserved along with their metabolic capacity in the long-term operation of critically important ecological processes (Lewis & Gattie, 1990).

COMPETITION BETWEEN BACTERIA FOR THE BIOFILM MODE OF EXISTENCE

The population structure that develops within a biofilm on a surface is determined initially by the success of a particular micro-organism in establishing a 'beachhead' on that surface in the presence of competing species. McEldowney and Fletcher (1987) demonstrated that the presence of one species on a surface inhibited the colonization of another species. Postma *et al.* (1990) demonstrated that colonization of soil particles by *Rhizobium leguminosarum* was more efficient when the soil particles were free of other microbes and no predatory flagellates were present. The presence of flagellates, however, increased the percentage of bacteria that were particle-associated relative to those that existed in the free-living population. Thus, grazing pressure promotes the biofilm mode of existence among soil microbial populations.

There is evidence that even after a bacterium establishes itself on a surface, displacement by other species can still occur. Biofilms of

Escherichia coli, *Ps putida* and *Ps aeruginosa* that developed on previously-sterilized activated carbon filters were modified by subsequent exposure to bacteria in tapwater (Rollinger & Dott, 1987). After exposure to unsterile running tapwater these three bacterial strains immediately decreased in abundance with no regrowth after 2 months. Competition for limiting nutrients was thought to be the reason for the displacement of the monitored species by autochthonous species in the tapwater. Similar results were observed by Camper *et al.* (1985) when *Yersinia enterocolitica*, *Salmonella typhimurium* and *E. coli* were exposed to autochthonous river water micro-organisms. It appears that established surface populations are constantly being challenged by newcomers introduced from the bulk water.

Competitive exclusion is not always observed, however. Studies by Cowan *et al.* (1991) have shown that there is little or no competition between different species for attachment sites on some surfaces. Neither a coryneform nor *Aeromonas hydrophila* were displaced by *Ps fluorescens* on glass surfaces exposed to nutrient-enriched lake water.

COOPERATION AMONG SPECIES FOR SITES ON A SURFACE

There are numerous examples where the presence of one species of bacterium promotes the attachment of another species of bacterium. *A. hydrophila* colonization was enhanced by the introduction of *P. fluorescens* (Cowan *et al.*, 1991). Addition of *Xanthomonas maltophilia* to the consortium increased the area of biofilm coverage on the surface. In another study, attachment of an *Acinetobacter* sp. promoted subsequent attachment by a *Staphylococcus* sp. and attachment of a coryneform to a surface promoted attachment of the *Acinetobacter* sp. (McEldowney & Fletcher, 1987). These studies demonstrate that the growth of some bacteria on surfaces is favored by a mutualistic or commensalistic interaction with other surface-associated species.

COAGGREGATION AS A STRATEGY FOR THE ESTABLISHMENT OF SPECIFIC BIOFILM POPULATIONS

According to Kolenbrander *et al.* (1985), the primary ecological determinant for successful colonization of a surface appears to be the selective adherence by the bacterium to that surface. Selective adherence may occur through intergeneric coaggregation, which is the recognition between surface molecules on two different bacterial cell types, resulting

in the formation of a mixed cell aggregate (Kolenbrander, 1988). Such interactions play a major role in bacterial attachment and colonization of tissue in the oral cavity. Nearly all human oral bacteria participate in intergeneric coaggregation (Kolenbrander, 1989). Intrageneric coaggregation has recently been demonstrated among strains of streptococci and actinomycetes. These species-specific reactions offer an explanation for their accretion on cleaned tooth surfaces and their dominance as primary colonizers (Kolenbrander *et al.*, 1990).

Two bacterial cell types that do not aggregate may have common partners, which becomes a coaggregation bridge (Kolenbrander *et al.*, 1985). *Bacteroides loescheii* and *B. intermedius* have many partners. Bridges that involve three or more cell types may be integral units in the consortium of cell types that contribute to plaque formation. Competition also has been demonstrated between two different cell types for the same partner.

A role for coaggregation in the formation of dental plaque has been suggested from a number of studies (Gibbons & Nygaard, 1970; Gibbons & van Houte, 1973; Kolenbrander *et al.*, 1985; Ellen & Grove, 1989). For example, when sucrose-grown cells of *Streptococcus mutans* or *S. sanguis* encounter cells of *Actinomyces viscosus*, a lectin on the surface of *A. viscosus* interacts with a carbohydrate of a polysaccharide on the *Streptococcus* cell surface (McIntyre *et al.*, 1978). Currently, there is an effort to identify the cell-surface molecules responsible for coaggregation and the genes responsible for these molecules. A greater challenge that begs attention is identification of the metabolic interactions among coaggregation species.

BIOFILM POPULATION SHIFTS IN RESPONSE TO ENVIRONMENTAL CHANGE

Changes in environmental conditions are known to affect biofilm populations. Even though biofilm bacteria are surrounded by an exopolymer matrix which provides some protection against the deleterious effects of moderate fluctuations in the external environment, metabolic processes of biofilm populations may be interrupted by large external disturbances. Recent studies by Osgood and Boylen (1990) in small woodland streams in the Adirondack Mountains of New York State indicated that despite the presence of a protective slime matrix, epilithic populations were affected by water chemistry. Total epilithic ATP levels correlated with the pH and acid-neutralizing capacity of the bulk water.

Transplant experiments demonstrated that epilithic ATP levels were

decreased by a shift from high (7·0) pH to low (4·9) pH. Tritiated thymidine uptake rates were generally lower in epilithic populations that developed in streams with low pH than those in streams with high pH. Compared with the more transient planktonic and more isolated sediment microbial populations in the streams, only the epilithic microbial population appeared responsive to chemical variations in the bulk water. Epilithic biofilms may therefore serve as useful biological sensors of water quality in lotic systems.

Microbial flora of fish

Microbial populations that reside as biofilms on exposed tissues of marine fishes serve as useful models to evaluate subtle responses of organisms to environmental stress. Cahill (1990) suggested that bacteria recovered from the skin and gills may be transient rather than resident populations on the fish surfaces. Thus, the microbiological quality of the water should be reflected on the exposed tissues of the fish that reside in that water.

This idea was tested in a study of microbial populations associated with the opaleye *Girella nigricans* in southern California coastal waters. The densities of the bacterial populations associated with the skin and gills of these fish varied directly with total concentrations of recoverable bacteria in the surrounding water. Fish collected in March from unpolluted tidepools in southern California which contained 5×10^3 colony forming units (cfu) ml^{-1} on Zobell's Marine Agar contained 4×10^3 cfu cm^{-2} on their skin and 3×10^4 cfu $gram^{-1}$ tissue on their gills, whereas fish collected from the same tidepools 8 months later in November contained 4×10^4 cfu cm^{-2} on their skin and 6×10^4 cfu $gram^{-1}$ tissue on their gills when the water contained 9×10^3 cfu ml^{-1}. The differences in the bacterial concentrations in the water at the two different sampling times were found to be significantly different, as were the bacterial densities associated with the skin of fish collected at these times. No significant difference was observed, however, between the bacterial densities on the gill tissue of fish collected at the two different sampling times. These results suggest that the bacterial population of the skin is more responsive to conditions of the surrounding water than the bacterial populations associated with gill tissue of the fish.

Fish transferred from pristine tidepools to polluted Los Angeles Harbor water developed significantly higher densities of aerobic heterotrophs on their gill tissue than fish transferred to 'clean' seawater (control) over a $6\frac{1}{2}$-week period (Fig. 1). The harbor water contained

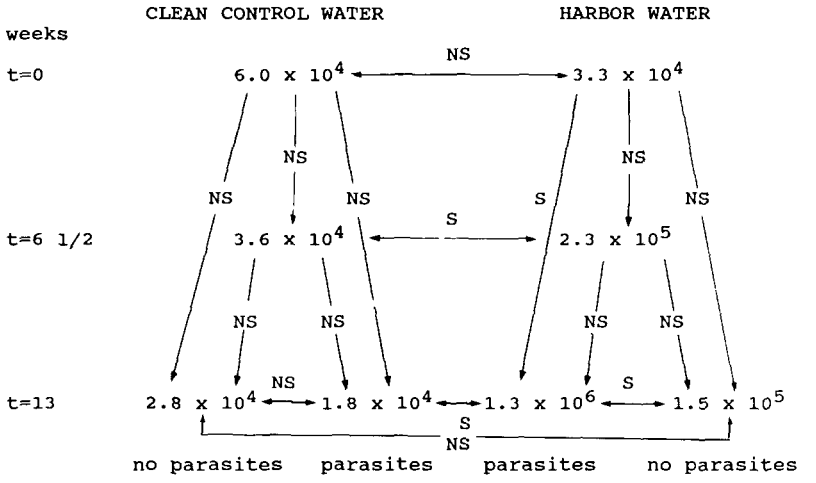

Fig. 1. Bacterial densities associated with gill tissue. Values reflect cfu gram^{-1} dry weight gill tissue. S refers to a significant difference at the 95% confidence level. NS indicates no significant difference at the 95% confidence level.

significantly higher bacterial densities than the clean seawater. Subsequent inoculation of the harbor water-exposed fish with the lungworm parasite, *Parafilaroides decorus,* to impose a stress on the immune system of the fish, produced a further significant increase in bacterial density on the gills after an additional 6½-week period relative to fish challenged with the parasite in clean water (Fig. 1). Bacterial densities on the skin increased significantly only after exposure to polluted harbor water and inoculation with the parasite (Fig. 2).

Pseudomonas spp. were the dominant type of bacteria associated with the skin of the fish, regardless of the time of year that the fish were sampled (Table 1). The population structure of the skin did not change over a 13-week period in clean water following capture. Likewise, no change in the population occurred when fish maintained in clean water were inoculated with the lungworm parasite (Table 1). However, fish transferred to harbor water exhibited a 29% higher frequency of *Pseudomonas* spp. on their skin 13 weeks after capture with 24% of the increase occurring within the first 6½ weeks of captivity (Table 1). Parasitic infection exerted little effect on the bacterial population structure of skin beyond that caused by the water. These results suggest that of the stress factors tested, the conditions of the harbor water are more important than parasite inoculation in causing changes in the bacterial population structure on the skin of the fish and that these stress

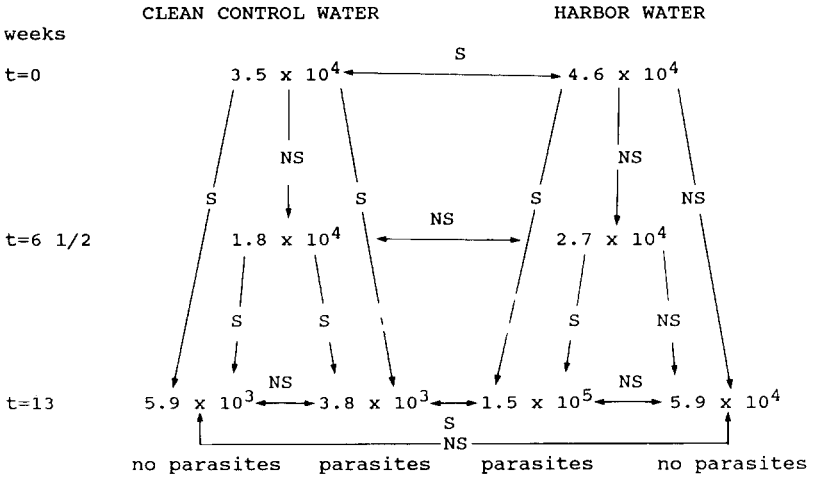

Fig. 2. Bacterial densities on skin. Values reflect cfu cm^{-2} skin surface area. S refers to a significant difference at the 95% confidence level. NS indicates no significant difference at the 95% confidence level.

factors do not appear to exert a synergistic effect on bacterial population structure on skin.

In contrast to what was observed on the skin, the bacterial biofilm population structure on gill tissue of fish maintained in clean water control exhibited a 13% increase in *Pseudomonas* spp. and a comparable decrease in *Vibrio* spp. when inoculated with the lungworm parasite (Table 2). Thus, bacterial populations on different tissues respond differently to the same stress factors under otherwise similar conditions.

Like that observed on the skin, gill tissue of fish transferred to and maintained for $6\frac{1}{2}$ weeks in polluted harbor water displayed an increase (33%) in *Pseudomonas* spp. and a decrease (17%) in *Vibrio* spp. (Table 2). *Flavobacterium-Cytophaga* also decreased (17%) in abundance under these conditions. Similarly, application of additional stress in the form of parasite inoculation caused no change in the bacterial population of the gills beyond that produced by the polluted harbor water (Table 2).

The trends observed in bacterial population dynamics on the tissues of fish exposed to the different water conditions appeared to be a subtle reflection of the health of the fish. Fish transferred to and maintained in polluted harbor water exhibited twice the mortality of fish transferred to and maintained in clean water control over a $6\frac{1}{2}$-week period (Fig. 3). Even greater differences in mortality were observed between fish maintained in clean and harbor water with longer exposure periods (13 weeks). Parasite inoculation increased fish mortality but not to the

TABLE 1

Bacterial Genera Associated with the Skin of *Girella nigricans* Transferred from Unpolluted Tidepools to Aquaria Containing Unpolluted Control Seawater or Untreated Polluted Harbor Water

Bacterial Genera[d]	*Percentage contribution*[a] *of bacterial genera*[b]							
	Time (weeks)[c]							
	T = 0[e]		*T = 6½*[f]		*T = 13 (No parasites)*[g]		*T = 13 (Parasites)*[g]	
	Polluted	*Control*	*Polluted*	*Control*	*Polluted*	*Control*	*Polluted*	*Control*
Pseudomonas	50	62	74	63	79	58	74	58
Vibrio	13	10	4	9	4	8	9	13
Aeromonas	8	5	9	4	0	8	4	4
Alcaligenes	13	10	4	17	8	13	4	17
Acinetobacter	4	0	0	4	0	0	4	0
Flavobacterium-Cytophaga	13	14	8	4	4	13	4	8
Total isolates examined	24	21	23	24	24	24	23	24

[a]Values represent the percentage contribution of bacterial genera on skin samples obtained from eight fish maintained in control water or polluted harbor water.

[b]Bacterial genera isolated from Zobel Marine Agar 2216 supplemented with 5% defibrinated sheep's red blood cells.

[c]Sampling periods of fish at 6½-week intervals.

[d]Bacterial genera recovered from the skin of fish.

[e]Fish collected from unpolluted tidepools ($T = 0$) and sampled prior to introduction into control water or harbor (polluted) water aquaria test systems.

[f]Fish sampled after 6½ weeks ($T = 6\frac{1}{2}$) of maintenance in control water or harbor (polluted) water aquaria test systems.

[g]Fish held in control and harbor (polluted) water aquaria for 6½ weeks ($T = 6\frac{1}{2}$) were separated into two groups. One group ($T = 13$ parasites) was force-fed larvae of *Parafilaroides decorus*, while the second group ($T = 13$ no parasites) received no parasites. Both groups were sampled after an additional 6½ weeks of maintenance in control water or harbor water aquaria test systems.

TABLE 2

Bacterial Genera Associated with the Gill Filaments of *Girella nigricans* Transferred from Unpolluted Tidepools to Aquaria Containing Unpolluted Control Seawater or Untreated Polluted Harbor Water

Bacterial Genera[d]	*Percentage contribution*[a] *of bacterial genera*[b]							
	Time (weeks)[c]							
	T = 0[e]		*T = 6½*[f]		*T = 13 (No parasites)*[g]		*T = 13 (Parasites)*[g]	
	Polluted	*Control*	*Polluted*	*Control*	*Polluted*	*Control*	*Polluted*	*Control*
Pseudomonas	50	57	83	61	75	54	71	67
Vibrio	21	24	4	17	8	21	4	8
Aeromonas	4	5	8	4	0	4	8	8
Alcaligenes	8	10	4	4	4	17	8	13
Acinetobacter	0	0	0	0	1	0	0	0
Flavobacterium-Cytophaga	17	5	0	13	8	4	8	4
Total isolates examined	24	21	24	23	24	24	24	24

[a]Values represent the percentage contribution of bacterial genera on gill filaments obtained from eight fish maintained in control water or polluted harbor water.

[b]Bacterial genera isolated from Zobel Marine Agar 2216 supplemented with 5% defibrinated sheep's red blood cells.

[c]Sampling periods of fish at 6½-week intervals.

[d]Bacterial genera recovered from the gill filaments of sampled fish.

[e]Fish collected from unpolluted tidepools ($T = 0$) and sampled prior to introduction into control water or harbor (polluted) water aquaria test systems.

[f]Fish sampled after 6½ weeks ($T = 6\frac{1}{2}$) of maintenance in control water or harbor (polluted) water aquaria test systems.

[g]Fish held in control and harbor (polluted) water aquaria for 6½ weeks ($T = 6\frac{1}{2}$) were separated into two groups. One group ($T = 13$ parasites) was force-fed larvae of *Parafilaroides decorus*, while the second group ($T = 13$ no parasites) received no parasites. Both groups were sampled after an additional 6½ weeks of maintenance in control water or harbor water aquaria test systems.

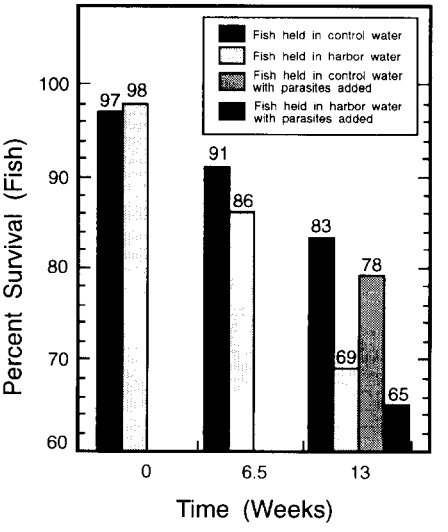

Fig. 3. Mortality of fish maintained in cleaned seawater and polluted harbor water.

extent observed after exposure to harbor water (Fig. 3). A synergistic effect was observed with water conditions and parasite inoculation on fish mortality (Fig. 3). The results suggest that variations in the density and population structure of bacterial biofilms colonizing those tissues of fish which are in contact with the external environment serve as a useful indicator of both lethal and sublethal stress to the host organism. Although biofilm population density and structure appear to reflect the health of the fish, it is not known whether the bacteria *per se* are directly responsible for the stress to the host or whether they simply reflect some other factors in the environment that debilitate the fish.

Studies using other fish species in other bodies of water have found that the bacterial populations on the host tissues do not reflect that of the surrounding water. Austin (1983) described the diversity of bacteria on the skin of turbot *Scophthalmus maximus* using numerical taxonomic techniques. The greatest diversity of bacterial taxa, totalling 25, were found from the skin of healthy fish. *Photobacterium agustum, Ph. logei, Alcaligenes faecalis, Ps fluorescens* and *Bacillus firmus* were found to be exclusively associated with healthy fish. The skin microflora did not closely reflect the types of bacteria found in the water. Mudarris and Austin (1988) found the highest numbers of aerobic heterotrophs on the gills of healthy turbot; $7{\cdot}0 \times 10^5$ cfu/gram wet weight gill tissue. All isolates were Gram-negative rods, many of which possessed appendages. The bacteria covered only 0·1–1·0% of the gill surface, primarily at

protected sites. Gill microflora was distinct from that of the surrounding water and from that of the skin. Thus, it appears that the tissues of the fish offer a distinctly different environment than the surrounding water and that the differences select for certain species of bacteria.

Human microbial populations

The urethra of healthy females is colonized primarily by *Lactobacillus* spp. which appear to protect the host and *Staphylococcus epidermidis* (Marrie *et al.*, 1980). *Bacteroides melaninogenicus* is the primary anaerobic bacterium present. The highest densities of these bacteria are in reproductive age females. The composition of the microflora changes with age. *Corynebacterium* spp., *Lactobacillus* spp. and coagulase-negative bacteria are common in premenarchal females. Reproductive age females have mostly *Lactobacillus* spp. Postmenopausal females have mostly *Lactobacillus* spp. and *Streptococcus* spp. with fewer *Corneybacterium* spp. present. Very few Gram-negative bacteria are found in healthy females. Higher concentrations of Gram-negatives are found in individuals with urinary tract infections. In some respect, this parallels the results obtained from the fish studies described above whereby the dominant urethral biofilms in healthy individuals do not represent those organisms that infect the bladder. Rather, the pathogens must first find a niche on the urethra before they can infect the bladder.

Like the influence aging has on biofilm community structure, treatments that interfere with the host's immune system also lead to a change in the microbial populations that colonize tissues. Immunocompromised patients develop an antibiotic-resistant population of bacteria that is not observed in healthy individuals (van der Waaij, 1989). Gram-negative enterobacilli are often more abundant in compromised individuals (van der Waaij, 1989). Antibiotic therapy is also thought to encourage the establishment of *Clostridium difficile*-associated colitis in the intestine (van der Waaij, 1989). In the absence of antibiotic therapy, the normal intestinal flora suppress colonization of *C. difficile* in the digestive tract. Saliva and intestinal mucosa provide continuous inoculum of indigenous microbes to the walls of the gut. Recolonization of the tissues following antibiotic treatment often requires repeated oral inoculation with indigenous species (Reid & Bruce, 1991).

Selective decontamination of Enterobacteriaceae and Pseudomonadaceae from the digestive tract has been achieved by selective antimicrobial drugs in immunocompromised patients in oncology treatment centers (Guiot *et al.*, 1981) and burn units (Manson *et al.*, 1987).

The composition of the indigenous intestinal microflora is under

immunoregulation. Gut-associated lymphoid tissue responds to antigens that come in contact with the tissue and respond by synthesizing and secreting IgA that limit colonization of mucosal epithelia by pathogenic bacteria. These antibody molecules control adsorption of the cells and their adhesions to the tissue surface. It has been proposed that bacteria which have surface antigens common to the host and enzymes that degrade nutrients at the surface, can freely colonize the tissue for the lifetime of the host, whereas, microbes that do not share common antigens with the host may stimulate IgA production which interferes with colonization and causes agglutination of the bacteria (Tomasi *et al.*, 1980).

Thus, a variety of perturbations either from the external environment or from within the host organism can lead to an alteration in the structure of the biofilm population that colonizes the host's tissues. Inevitably, a condition that disturbs the normal biofilm population structure will facilitate the colonization of pathogenic species that increase the likelihood of disease in the host. The manifestation of disease is most often reflected in the biodeterioration of host tissue.

BIOFILM POPULATION STRUCTURE AS A DETERMINANT OF TOOTH AND GUM DISEASE

The types and abundance of bacterial species present in plaque change as the clinical condition of the host changes from normal health through gingivitis to advanced stages of periodontitis (Kolenbrander *et al.*, 1985). The predominant flora initially observed on the tooth surface are gram-positive bacteria such as streptococci and actinomycetes. Subgingival bacteria found in healthy gingiva form coaggregates with other bacteria associated with healthy gums. Secondary colonizers include *Haemophilus*, *Veillonella* and *Peptostreptococcus*. The normal oral flora is quite stable in healthy individuals. This population structure is destroyed, however, during severe illness (Bagg, 1990). The population changes to mainly gram-negative bacteria during the onset of gingivitis. No one particular bacterium appears to be clearly identified as the causative agent of the disease. However, the bacteria most commonly associated with periodontal disease are *A. actinomycetemcomitans*, *B. intermedius*, *B. gingivalis* and *Capnochtophaga gingivalis*. Several other genera of bacteria which are in low densities in the gingivitis stage are often more abundant after onset of periodontal disease. *Eubacterium nodatum* appears only after clinical symptoms of periodontal disease are observed. Recently, spirochaetes were isolated from plaque of patients with gum disease

(ulcerative gingivitis) but not in a group of healthy controls (Riviere *et al.*, 1991). Bacteria in more advanced stages of gum disease tend not to recognize early colonizers but rather coaggregate with other bacteria found in the diseased gum. The changes in biofilm population structure over time are possibly due to host resistance to a particular consortium (Kolenbrander *et al.*, 1985). The species changes that occur with biofilms that develop on the tissues in the oral cavity support the concept that the pathogenic state is the product of dynamic selective pressures on microbial populations (Finlay & Falkow, 1989).

BIOFILM POPULATION STRUCTURE BASED ON SPECIFIC METABOLIC CAPACITIES OF BACTERIA

A major mechanism of subgingival plaque formation in the gingival sulcus is the selection of certain bacteria based on their metabolic capacity rather than their affinity to surfaces (Rolla *et al.*, 1991). Development of anaerobic conditions encourages proliferation of anaerobic gram-negative bacteria. Using micro-electrodes, Lewandowski *et al.* (1991) demonstrated that the oxygen concentration through a 0·7 mm-thick mixed species biofilm decreased from 8 mg/liter in the bulk aqueous phase to 1·2 mg/liter at the biofilm–bulk water interface to zero at the base of the biofilm. The dissolved oxygen diffusion coefficient in the biofilm was calculated to be $1{\cdot}76 \times 10^{-5}$ cm^2 s^{-1} and the dissolved oxygen flux across the biofilm–bulk water interface was $1{\cdot}02 \times 10^{-6}$ mg s^{-1} cm^2. These results suggest that an actively respiring aerobic biofilm can reduce the oxygen concentration to sufficiently low levels at the base of even biofilms that are only tens of micrometers thick to support conditions for anaerobic bacterial growth.

Dental plaque harbors high numbers of *S. sanguis* and low numbers of *S. mutans* in the absence of sucrose (Gibbons & van Houte, 1971). Although sucrose is not essential for the colonization of hard dental tissue by *S. mutans*, it does promote its growth in plaque. Sucrose-enhanced colonization of tooth enamel by *S. mutans* is of significance to the development of caries following plaque formation (Rolla *et al.*, 1991). Formation of a polysaccharide matrix in the presence of sucrose has been suggested to concentrate acid by-products of cell metabolism which demineralize the outer enamel layer of the tooth. The enzyme, glycosyl transferase, is responsible for the synthesis of the polymer as well as adhesion of the plaque to the tooth. In the presence of the enzyme, the glucan provides a matrix for the binding of other types of acid-producing bacteria.

CONCLUSIONS

The biofilm mode of growth among micro-organisms in nature appears to have evolved, in part, to buffer individual cells from widely varying conditions in the external environment. The consequence of having a protective exopolymeric matrix around the cells could prove more detrimental than exposure to the external insult due to the entrapment of toxic metabolic products were it not for the fortuitous associations that develop among adjacent physiologically-distinct cells within a biofilm. The benefit to one species by the removal of its metabolic products by another strategically-positioned species in the biofilm forms the basis for a complex interdependence that controls the microbial population structure in a dynamic environment. The vectorial metabolism carried out at the community level by physiologically-distinct, spatially-oriented biofilm micro-organisms is likely to be as important for the survival of these microbes as that contributing to proton gradients and group translocation for energy production and utilization at the cellular level.

To date, we understand only a few of these structured interactions. Remarkably, even this limited knowledge has permitted our exploitation of some of these associations in our technology. It is not unreasonable to assume therefore that the untapped metabolic potential of those biofilms which we have yet to discover will provide solutions to many of our medical, industrial and environmental problems. Disruption of microbial biofilm population structure through selective chemical or physical treatment may be all that is necessary to control biofilm activities in these environments.

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