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Significance of Bacterial Surface-Active Compounds in Interaction of Bacteria with Interfaces

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INTRODUCTION AND TERMINOLOGY

"Lipids tend to be the forgotten components of microorganisms" (150). The same is true for a subclass of microbial lipids, the microbial surface-active compounds (SACs). The difference between lipids and SACs is based on the ratio of the hydrophobic to the hydrophilic regions. This relation is expressed for synthetic surfactants as the hydrophilic/lipophilic balance value and can vary over a wide range. The three general characteristics of surfactants are enrichment at interfaces, lowering of interfacial tension, and micelle formation. For example, some synthetic surfactant structures which have been used in microbial adhesion studies are presented (Fig. 1). To compare surfactants, the surface or interfacial tension is used as a measure of effectivity. The concentration at which surfactants in solution start to form aggregates is defined as the critical micelle concentration. The critical micelle concentration is used as a measure of efficiency. These characteristics are also applicable to biosurfactants; however, not all of them may be applicable to polymeric microbial SACs.

As the name "surface-active compound" suggests, these substances tend to interact with interfaces. An interface is defined as a phase boundary between two phases in a heterogeneous system. For all interfacial systems, it is known that organic molecules from the nonsolid phase immobilize at the solid interface. There they eventually form a film known as conditioning film, which will change the properties of the original surface (106). In this way, the conditioning film may influence the interaction of bacteria with the interface. The molecules composing the conditioning layer include small and polymeric compounds such as lipids, proteins, complex polysaccharides, and humic substances (27). Those organic molecules conditioning the surface will change the wettability and surface charge of the original surface as determined via contact angle and free surface energy measurement, respectively. Substratum surface properties will determine the composition and orientation of the molecules conditioning the surface during the first hour of exposure. After about 4 h, a certain degree of uniformity is reached and the composition of the adsorbed material becomes substratum independent (104, 106, 128, 135). More recently, it was shown that the predeposition of a

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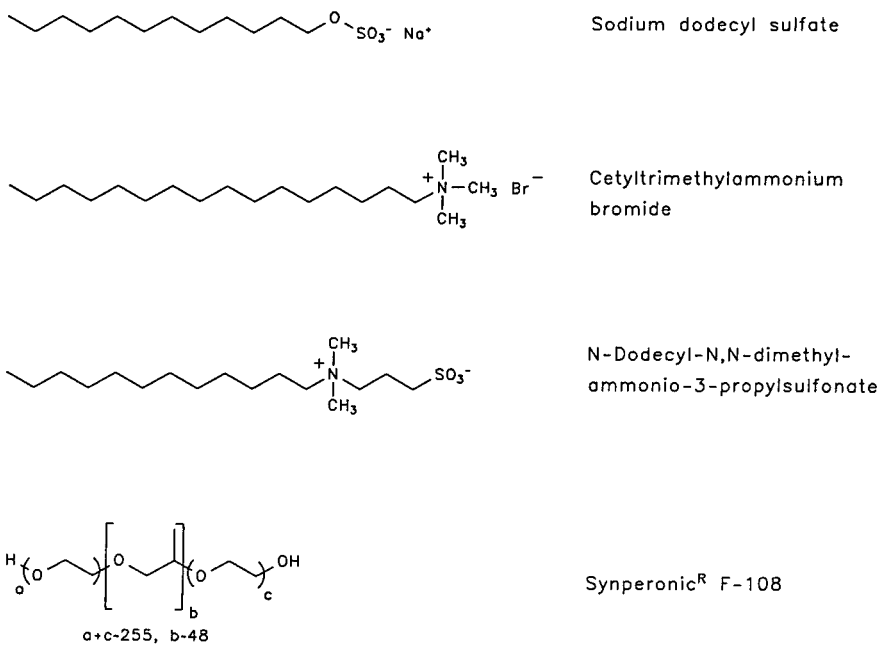

FIG. 1. Examples of various types of synthetic surfactants used in microbial adhesion studies: SDS is anionic; cetyltrimethylammonium bromide (CTAB) is cationic; *N*-dodecyl-*N*,*N*-dimethylammonio-3-propylsulfonate (Sulfobetaine 3-12) is amphoteric; block copolymer surfactant (Synperonic F-108) is nonionic. Courtesy of G. Kopf.

conditioning film from waters with low carbon content strongly affects the interaction of bacteria with interfaces (169, 170). In an analogy to organic conditioning films, microbially produced SACs may interact with interfaces and will affect the adhesion and deadhesion of bacteria.

Generally, surface-active properties are very important for a huge number of natural and technical processes taking place at interfaces. In a recent book, the key term related to all these processes, wettability, has been elaborated in detail and various theoretical and applied approaches are described (172). Also, microorganisms in nature tend to have a preference for interfaces, where they perform both wanted and unwanted activities (113). The wettability of interfaces may be a signal for organisms which have a sessile stage as part of their life cycle (122). This fact eventually created a microbiology research area concerned with microbial adhesion and biofilm development. The continuously growing number of publications in the field of microbial interaction with interfaces, as well as special conferences on this subject, underlines this statement. A substantial number of these reports investigated the molecules of the microbial cell surface which might be involved in the interaction of cells with interfaces. The molecules described so far are mainly proteins and polysaccharides. Apart from these two classes of polymers, microorganisms have the potential to produce other molecules, such as biosurfactants, amphiphilic polymers, and polyphilic polymers which are ideally suited to interact with interfaces.

Biosurfactants are defined as low-molecular-weight surfactants, e.g., glycolipids and peptidolipids. For high-molecular-weight SACs with a hydrophobic region at one end of the molecule, e.g., lipopolysaccharides and lipoteichoic acids, the term "amphiphilic polymers" is more suitable. If the hydrophobic groups are distributed across the entire polymeric molecule, the SACs are identical with hydrophobic comb-type polymers. In this case, they may be called polyphilic polymers; examples include hydrophobic polysaccharides and emulsan (Table 1).

Another key term for most interfacial biological processes is hydrophobicity. There are extended reviews on microbial cell surface hydrophobicity and hydrophobic interactions in bacterial adhesion (37, 159). However, explanation and understanding of the hydrophobic effect remain difficult. Therefore, the summary definition of hydrophobicity by Duncan-Hewitt (38) may be cited. "A working definition of hydrophobicity is elusive. It is formally defined in terms of the hydrophobic interaction process, in which two solute molecules are brought together from infinite separation. The free energy for this process is divided into two terms, one that quantitates the direct force between the two solute molecules and an indirect part that is mainly a function of the solvent (the hydrophobic

TABLE 1. Terminology of microbial SACs as used in this review

Term	Definition	Examples
Biosurfactant	Low-molecular-weight surfactant	Glycolipids, peptidolipids
Amphiphilic polymer	High-molecular-weight surface-active polymer with one hydrophobic region at one end of the molecule	Lipopolysaccharides, lipoteichoic acids, lipoglycans
Polyphilic polymer	High-molecular-weight surface-active polymer with hydrophobic groups distributed across the entire molecule identical to hydrophobically modified, comb-type polymers	Hydrophobic polysaccharides, emulsan

interaction)." Nevertheless, the present review is not intended to elaborate the various aspects of hydrophobic interaction. For further information, the reader should consult two detailed books on hydrophobicity (14, 185).

Some of the other terms commonly used in describing microbial interaction with interfaces should be briefly defined. The terms "adsorption" and "desorption" are usually reserved for the interaction of molecules with interfaces where the type of interaction may be known. For microbial interactions with interfaces, the terms "attachment" and "detachment" are used if the microorganisms are firmly and irreversibly attached. By certain biological mechanisms or physical-chemical treatments, the microorganisms may then become detached. If the microorganisms are in the reversible state of interacting with an interface, the terms "adhesion" and "abhesion" are more appropriate. However, to avoid confusion, the term "deadhesion" is employed in this article instead of "abhesion."

In this paper, the different types of microbial SACs are introduced and reference is made to the main literature. Then the influence of SACs on microorganisms is described by looking first at the effect of synthetic SACs and second at the effect of microbial SACs. In this outline, a discussion of the synthetic surfactants and the general influence that surfactants may have on microorganisms at interfaces is included. Next, the physiological role of microbial SACs, as far as it is known, is briefly elaborated. The discussion on the role of microbial SACs in the interaction of microorganisms with interfaces is divided into two parts. The first part concerns adhesion processes, and the second deals with deadhesion processes. The interaction of hydrophobic cells with hydrophobic and hydrophilic interfaces and that of hydrophilic cells with hydrophobic and hydrophilic interfaces are treated separately. In addition, it is necessary to differentiate between low-molecular-weight biosurfactants and high-molecular-weight polymeric SACs. Finally, because the location of these compounds is also important, their presence at the microbial cell surface or at the interface is taken into account. The phenomenon of gliding, as a continuous deadhesion in a two-dimensional system, is discussed in a separate section. In the final section, speculation on the possible regulation of microbial cell surface hydrophobicity via microbial SACs is offered.

TYPES OF BACTERIAL SACs

Microorganisms are able to synthesize a wide range of different SACs, which are discussed in several reviews (30, 62, 92, 154, 210, 211) as well as in two books (90, 91). The various SACs of microorganisms can be distinguished in terms of different criteria. The size of the molecules can span a wide range from low-molecular-weight surfactants through polymeric surfactants up to particulate surfactants (154). Another criterion for categorizing SACs of microorganisms is the biochemical nature of the molecules such as fatty acids, lipids, bacteriocines, peptides, and polysaccharides (210). A further way to classify SACs of microorganisms is by the nature of the hydrophilic part of the SACs such as the carboxylate group of fatty acids, the glycerol of glycerolipids, the carbohydrate of glycolipids, and the amino acids of peptidyl lipids (30). Other authors distinguish between different locations of SACs in terms of intracellular, cell surface, and extracellular pool (91). Furthermore, SACs of microorganisms can be grouped by the species of the producing organisms. The SACs of microorganisms may also be divided according to the type of carbon source used to produce them, such as hydrocarbons, water-soluble molecules, or both (62). Some structural examples of biosurfactants produced on nonhydrocarbon substrates are given for illustration (Fig. 2).

There are other reviews focusing on diverse aspects of microbial SACs, such as biosynthesis (209), physical chemistry (211), and commercial applications (46, 90), especially for use in microbially enhanced oil recovery (125, 208).

In addition to the already mentioned microbial SACs, there are other molecules that are amphiphilic and surface active, which are often forgotten in a discussion of microbial SACs. These molecules represent part of the outer bacterial layers as structural compounds and can be divided into bacterial cell surface amphiphiles (202) and polyphilic polymers (135). The bacterial amphiphiles are common in both gram-negative and gram-positive bacteria. Typical examples for gram-negative bacteria are lipopolysaccharides (107, 153, 198) and the enterobacterial common antigen (93). Examples for gram-positive bacteria are lipoteichoic acids (88), lipoglycans (182), lipomannan (103, 147, 183), *Actinomyces* amphiphile (202), and some other amphiphiles (76, 151, 205). For lipoteichoic acid, it was demonstrated that the acylated form is excreted into the medium and forms micelles with a critical micelle concentration of 1 to 10 μg/ml (201). A second class of cell surface amphiphiles contains the lipoproteins, which are anchored in a variety of ways via their lipid part in the outer cell layers of gram-negative and gram-positive bacteria (149).

The second and very much neglected group of SACs contains the microbial polyphilic polymers, for example, polysaccharides containing either deoxy sugars or other hydrophobic substituents such as acyl, methyl, or other groups. An exception is the *Acinetobacter calcoaceticus* RAG-1 polysaccharide, emulsan. This is the only polysaccharide found so far that carries fatty acids distributed over the entire molecule, resulting in a hydrophobically modified, comb-type polymer. The biological role of emulsan is discussed in a later section. The polyphilic polymers differ from the cell surface amphiphiles in that they do not carry one single lipid part at one end of the molecule. Because they carry the hydrophobic groups over the entire length of the molecule, the properties of the molecule are the summation of the properties of the hydrophobic groups on each repeating unit. Typical examples of sugars within these polyphilic polysaccharides are the 6-deoxy sugars rhamnose and fucose, as well as *N*-acetyl hexosamines. However, there are other deoxy sugars in microbial polysaccharides, which may be responsible for their hydrophobic character (85). The unusual behavior of these polyphilic polysaccharides has been described by several authors. The surface tension of highly substituted microbial polysaccharides with a high deoxy sugar content revealed values lower than 50 mN/m (184). The characterization of the polysaccharides of a marine *Pseudomonas* strain showed that one of the two polysaccharides produced was soluble in 90% aqueous phenol, 80% methanol, and 80% ethanol (28). These are concentrations of organic solvents at which polysaccharides normally precipitate. An investigation of the polysaccharide of an adhesive hydrophobic *Rhodococcus* species also revealed surface-active properties. The surface activity could be demonstrated directly by using a tensiometer as well as indirectly by static light scattering (138). In all of these publications, the surface activity of the polysaccharides was explained by the presence of deoxy sugars and *O*-acetyl groups. Some of the 6-deoxy sugar-containing polysaccharides were isolated as polymeric emulsifiers (82, 133, 139, 155), and others were isolated as drag-reducing polymers (15, 155, 163). Chemically derivatized polyphilic polysaccharides are commercially available and have potential industrial applications. There are even screening strategies to isolate microorganisms producing 6-deoxy sugars. These sugars are important as sub-

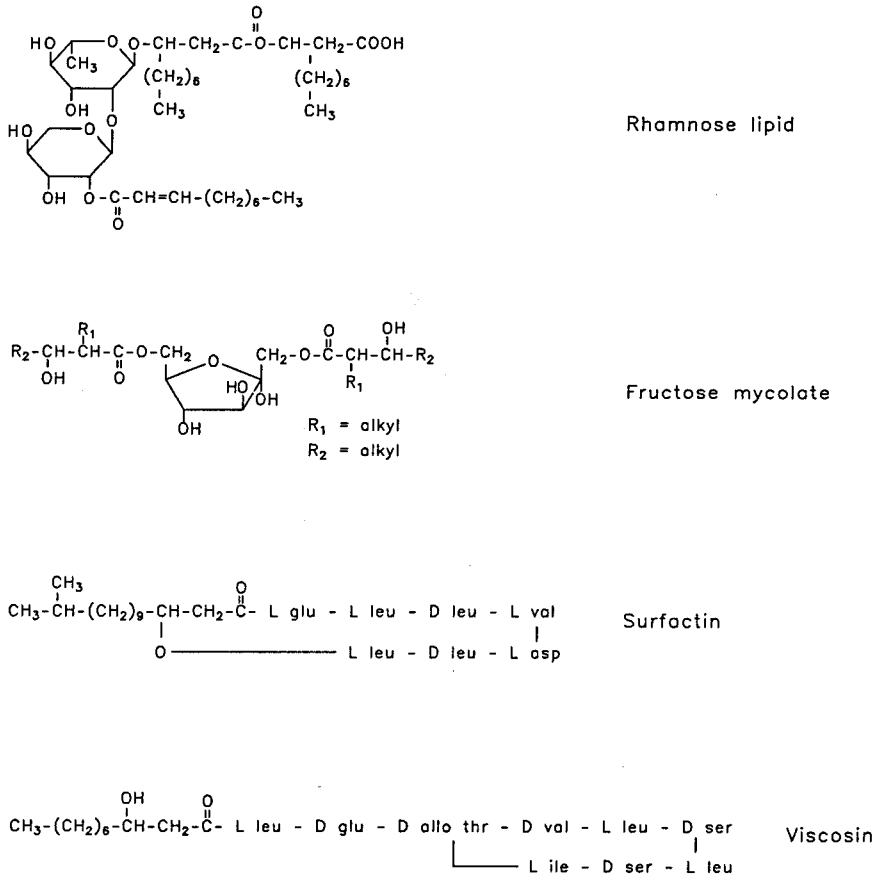

FIG. 2. Microbial SACs produced on nonhydrocarbon substrates. Rhamnolipid and fructosemycolate are glycolipids; surfactin and viscosin are peptodolipids. Courtesy of G. Kopf.

strates in the chemical synthesis of flavoring agents and are known to alter the basic properties of water (60, 61). The term "polyphilic polymer" or "hydrophobic polymer" must not be restricted to polysaccharides but may be used for proteins with similar properties.

SYNTHETIC SACs AND BACTERIA

In Solution

Synthetic surfactants in solution have been used in a large variety of experiments investigating the microbial cell surface as well as the interaction of microorganisms with interfaces. All these studies were designed to examine the cell surface properties of microorganisms, the possible hydrophobic interactions with interfaces, or the potential application of synthetic SACs as cleaning compounds.

Sodium dodecyl sulfate. Sodium dodecyl sulfate (SDS) has frequently been used to study the effects of surfactants on bacteria. In experiments with oral streptococci, it could be shown that hydrophobic bond-disrupting agents including SDS inhibited the adhesion of the bacteria to hydroxylapatite (130). SDS and sugars were found to inhibit the coaggregation of *Actinomyces viscosus* and *Streptococcus sanguis*. McIntire et al. (121) speculated that the lectin site possesses an affinity not only for carbohydrates but also for nonaromatic amphipathic molecules. The adhesion of dental plaque bacteria to buccal epithelial cells is also dependent on hydrophobic interactions. It was shown that the adhesion of bacteria can be inhibited up to 90% by SDS and also by saliva, suggesting that hydrophobic interactions mediate adhesion (160). Microbial oxidation of sulfur compounds is dependent on the adhesion of *Thiobacillus* species to hydrophobic elemental sulfur. In an experiment with *Thiobacillus albertis*, SDS was identified as a compound leading to nearly complete deadhesion of cells from inert surface (20). In a series of experiments, the biodegradable anionic surfactant SDS was used to investigate the interaction of bacteria with river sediment. The results showed a correlation between SDS biodegradation and an increase of adhesion which reversed after completion of biodegradation. It could be demonstrated that during biodegradation, the cell surface of a *Pseudomonas* strain became increasingly hydrophobic, a change which could be reversed by the removal of the primary intermediate of SDS biodegradation (110, 111). This effect can be summarized as an acceleration of biodegradation in the presence of sediment as a result of stimulation of bacterial adhesion by surfactants (112).

Quaternary ammonium compounds. Several studies employed cationic surfactants such as quaternary ammonium compounds (QACs). QACs bind by chemisorption to the cell surface of bacteria because the microbial cell surface at physiological pH is negatively charged. Thus, QACs do influence the zeta potential of bacteria (171). QACs are also known to be water contaminants. Their use as fabric softeners in households and antistatic agents in industry leads to concentrations of up to 25 mg/liter in river water. In the environment, they also seem to enrich at interfaces. A study on bacterial and

plankton activity in the river Rhine revealed an inhibition starting at a concentration of 1 mg/liter (191). A study of the effects of QACs on lake microbial communities showed that the microbial communities had adapted to the toxic effect and become more active in biodegradation of the QACs (193). The resistance of *Pseudomonas aeruginosa* to QACs was reported in another study. The tolerance correlated with changes of the outer membrane fatty acid composition and the ultrastructure (81). For *Acinetobacter calcoaceticus*, it was demonstrated that increased resistance to QACs allowed the isolation of mutants with enhanced capsule production (173). QACs have been also reported to enhance the biological inactivation of adhering *Listeria monocytogenes* by listeriaphages (161). Other studies with cationic surfactants used cetylpyridinium chloride. This compound enhances microbial adhesion to hexadecane and polystyrene. The increased adhesion was explained by the binding of cetylpyridinium chloride via electrostatic interactions to the cell surface, resulting in an increasing cell surface hydrophobicity (55). It was suggested that this observation could be applied to the development of new, more effective oil-water mouthwashes (56).

Various surfactants. Various surfactants have been applied in solution to study their effect on bacterial adhesion. Marine *Vibrio* showed, under starvation conditions, a similar response to a surface as well as to a surfactant, e.g., Tween 85. This triggering effect of surfactants clearly indicates a relation of the three participating components: bacteria, surfaces, and surfactants (70). The effect of Triton X-100 on adhesion of marine bacteria to hydrophobic and hydrophilic surfaces revealed differences depending on the free surface energy of the substratum. The surfactant inhibited the adhesion to hydrophobic but not to hydrophilic surfaces. It was suggested that there are surfactant-independent mechanisms for the adhesion to hydrophilic surfaces (144). Triton X-100 was used to compare the adhesion of *Azospirillum brasilense* to polystyrene and wheat roots. For both surfaces, a reduced adhesion was observed at surfactant concentration of 10 μl/liter (9). In studies of the adhesion of bacteria to epithelial cells, an inhibition by surfactants and hydrocarbons could also be shown (41, 45). As a fungal pathogen, *Candida albicans* has been subject to several investigations to examine its surface properties. The surface-active properties of yeast cells were demonstrated by using various surfactants. Furthermore, a strain-dependent influence of surfactants in the adhesion to hydrophobic plastic material could be established (64, 86, 87). Surfactants were successfully applied in the development of methods to improve the recovery of bacteria from environmental samples (50, 207). Similarly, surfactants have been included in cleaning strategies for the removal of biofilms in technical systems. The most efficient mixture was an anionic detergent-denaturant combination (200). These findings were confirmed in another study, in which commercial cleaning solutions were applied to remove biofilms from ultrafiltration membranes (48). To understand the influence of electrostatic and hydrophobic interactions on bacterial adhesion to polystyrene, a variety of electrolytes and surfactants were examined. It was found that the largest degree of deadhesion was produced by treatment with SDS or Tween 80, but evidence for both electrostatic and hydrophobic interactions was found (120). To further clarify the identity of interfacial forces, the influence of chemicals on adhesive polymers has been investigated. Treatment with Tween 20 showed an expansion of the adhesive polymers, indicating that hydrophobic interactions are significant for polymer conformation (114). The biodegradation of hydrophobic compounds has been the subject of numerous studies (see reference 105 for list of references). In general, a beneficial effect of surfactants on biodegradation has been observed. However, in some studies, a negative effect was found. This could be explained by the prevention of bacterial adhesion to the hydrophobic substrate by the surfactant or by the unavailability of substrate within the micellar phase (194). Surfactants were also found to inhibit swarming and gliding of *Cytophaga* strains by blocking the adhesion of the bacteria to the substratum (23).

Immobilized on Surfaces

Surfaces conditioned with synthetic surfactants were used mainly in studies aiming at developing nonfouling coatings and materials. The development of such a surface would have a large impact in a variety of fields, some of which are outlined below. Furthermore, it would have an enormous economic significance for technical and medical applications in which interfacial processes are critical.

Insolubilized quaternary ammonium compounds. QACs are cationic surfactants. As early as 1972, they were reported to be very effective substratum-bound antimicrobial compounds. Because their effect was very promising in the development of antifouling surfaces, they have been the subject of several studies. QACs have been used as organosilicons bound to surfaces. It could be shown that the antimicrobial activity was not attributed to the desorption of the chemical but rather to the surface-bound chemical (75). Similar findings have been reported by testing various algae exposed to the hydrolysis product of 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride chemically bound to a substratum (196). To evaluate the rate of kill of nonleaching antimicrobial QACs, surface kinetic test methods were developed with *Escherichia coli* as a model organism. This work resulted in the development of commercial products as well as in several patents (76, 180). Later, the effects of QACs after immobilization onto ion-exchange resins and porous glass were investigated. In comparison with uncharged model compounds, the electrical charge of QACs is necessary for it to be active against microorganisms (128). Furthermore, related compounds such as alkyl pyridinium iodides have been used to test for their antimicrobial activity. By examination of various bacterial strains, it was shown that the activity of the compound was dependent on the type of bacteria and the conditions employed. A crucial problem was the durability of the antimicrobial activity, which decreased remarkably within 24 h (127, 129). In experiments with enveloped viruses, it was demonstrated that their inactivation is caused by the disruption of the lipid envelope. These findings may not be applicable to bacteria because of the different architecture of their cell envelopes (188, 190). Nevertheless, the nature of the interaction of QACs with microorganisms which leads to cell death remains unclear. To my knowledge, no further work has been published.

Insolubilized block copolymer surfactants. Other promising compounds in the development of antifouling coatings are the so-called block copolymer surfactants. These compounds are polymeric surfactants which adsorb via their hydrophobic part to hydrophobic surfaces while the hydrophilic segments extend free into the water. It was suggested that the protruding hydrophilic chains form a steric barrier which prevents protein, bacterial, or eucaryotic fouling of the coated surfaces.

A study with different surface-immobilized biological polymers and synthetic block copolymer surfactants showed a significant effect of only Brij 56 on bacterial adhesion to hydrophobic but not hydrophilic surfaces. This nonionic surfactant (polyethylene oxide-10-cetyl ether) caused a >99% reduction in adhesion of a marine *Pseudomonas* species to polystyrene (71). In a later report (72), this effect was verified for other

ethoxylated surfactants such as Synperonic A, NP, Brij, and the Myrj series. The effect of the Procetyl series (alcohol propoxylates) was explained by the low solubility, the longer adsorption, and the slow release from the surface. In general, it was found that surfactants with either polyethylene glycol or polypropylene glycol chains can inhibit bacterial adhesion to hydrophobic surfaces (72). This antiadhesive effect was tested in the marine habitat by using the Synperonic PE series. These surfactants showed an excellent short-term effectiveness, whereas in long-term tests the effect was poor. This was explained by leaching of the surfactant, abrasion by sand, interference with natural polymers, the existence of specific bacterial populations able to overcome the copolymer barrier, and biodegradation of the surfactant (18).

Microbial adhesion and its prevention in the medical field are a key factor for successfully applying biomaterials. Therefore, several groups evaluated the effect of block copolymer surfactants in preventing the adhesion of bacteria to a variety of biomaterials. Usually, there was at least a 95% reduction of adhesion. Again, the effect was explained by the steric hindrance of the hydrophilic chain of the surfactant (19, 35, 66, 142). In addition to the above microbiological studies, there are numerous reports investigating the adsorption of proteins. The development of nonfouling biomaterials for medical applications has recently become one of the hot spots of scientific interest. To achieve a protein-resistant biomaterial, block copolymer surfactants have frequently been used to modify the biomaterial surface (6, 98, 99, 107, 115, 162, 175, 197).

Miscellaneous Effects

Apart from the above-mentioned effects, synthetic SACs have been used in only a few other very diverse investigations. The effect of Tween on the cell morphology and growth of *Lactobacillus* species was investigated. It could be demonstrated that the bacteria require Tween for aerobic growth under limiting glucose in a chemostat. This observation was interpreted in terms of lipoteichoic acid formation, because the concentration of Tween was inversly related to the cellular lipoteichoic acid concentration (78). Synthetic surfactants also support the activity of ligninase (100, 101) and have been described as general stimulants of enzyme production by microorganisms (151). The affected cellular compartment seems to be the cell membrane, where a change in the fatty acid composition was found. For *E. coli*, it has been reported that foaming produced by a surfactant can promote the conjugal transfer of plasmids. However, no explanation for this effect was provided (178). In another study, a nonionic surfactant was used to investigate the hydrophobicity of bacterial cells. The concentration of adsorbed surfactant could be correlated with the degree of cell surface hydrophobicity (140). In a recent paper, the interaction of bacteria with well-defined substrata of alkanethiol self-assembled monolayers was described. This approach offers the possibility to further characterize the chemical parameters of bacterial adhesion by using interfacially active compounds (203).

BACTERIAL SACs AND BACTERIA

Physiological Roles

The common view attributes only one role for microbial SACs, i.e., the growth of microorganisms on hydrocarbons. Most publications in this field discuss biosurfactants with respect to the growth of bacteria on water-insoluble carbon sources. The models for uptake of hydrocarbons consider the roles of dissolved molecules, contact of the cells with large oil droplets, or contact with fine oil droplets (68).

In addition to the role of bacterial SACs for growth on hydrocarbons as a carbon source, some other functions are mentioned in two review articles. Rosenberg (154) suggested that the diversity of structures and functions is a general property of microbial SACs and clearly stated that "It is unlikely that they all serve the same function." He discussed a function in adhesion with the adhesion to hydrocarbons as a special case, a function in the emulsification of water-insoluble compounds as substrates, and a function in deadhesion from interfaces. Furthermore, he mentioned a role in gliding and cell-cell interaction. Haferburg et al. (62) also made clear that the exact physiological functions of most microbial SACs remain unclear. They discussed microbial SACs mainly in terms of hydrocarbon assimilation and biocide activity. However, they also suggested a possible role in gliding of bacteria and in wetting of interfaces.

The biocidal activity of microbial SACs is closely related to the lipid moiety of the molecules. The consequences of the interaction of these compounds with eucaryotic cells are well known and include pyrogenicity, lethal toxicity, immunogenicity, mitogenicity, and other molecular effects (202). The lytic activity of biosurfactants produced on media without hydrocarbons was also described as a selection criterion for microorganisms producing SACs (126).

The hydrocarbon-water interface is not the only naturally occurring hydrophobic interface. Other important hydrophobic interfaces include the air-water interface, the aerial parts of plants with their hydrophobic cuticle and wax layers, the chitin skeleton of arthropods, and coal, tar, or elemental sulfur interfaces. The microbial degradation of leaves and chitin is essential for the recycling of the organic material in nature. The nonliquid hydrophobic interfaces also indicate a role for microbial SACs in growth on these substrata and their utilization as a substrate. The water-gas or air interface represents a universal hydrophobic interface not only in aqueous systems such as rivers, lakes, and the sea but also in soils and sediments. Hydrophobic molecules tend to accumulate at this interface and are an attractive nutrient source for interfacial active bacteria. Microbially produced SACs seem to be ideal molecules to enhance the interaction with all of these natural hydrophobic interfaces.

Another important issue is the identity of microbial cell membrane, cell wall, and cell surface turnover products with respect to microbial SACs. For gram-positive and gram-negative bacteria, amphiphilic compounds which carry a hydrophobic moiety are known. This lipid part may be structural and serve as a lipid anchor in the membrane, or it may be a part which is temporarily attached to a polymer for transfer through the membrane (21, 182). Bacterial cell walls and peptidoglycan have been the subject of several reviews (89, 165, 189). As the bacterial cell wall determines the shape and mechanical properties of cells, the synthesis and turnover of cell walls are most critical during the growth of cells. Again, gram-positive and gram-negative cells should be treated differently because of the variation in cell wall thickness and the presence or absence of an outer membrane. By definition, cell wall turnover does not require growth, and it is not essential that the turnover products be released into the environment. Generally, the cell wall turnover products that are released by hydrolases from the murein are recycled by the cell (31, 36, 67). Finally, there are cell surface turnover products which represent the outermost surface layer of bacteria. These compounds may include peptidoglycan in gram-positive cells but also other molecules such as proteins and polysaccharides (36, 149, 199). Nearly all of the

bacteria in the environment are covered by extracellular polymeric substances. Because of the response of the bacteria to environmental changes, these extracellular polymeric substances may be released from the cell surface. At least some of the microbial SACs may fall in the category of cell surface turnover products which are released into the environment under certain growth conditions.

Other Observations

Only limited information about the influence of microbial SACs on microorganisms themselves is available. However, careful examination of the literature reveals a few hints about the possible function of these compounds.

The presence of the polyphilic polymer emulsan enhanced the tolerance of *Acinetobacter calcoaceticus* to the toxic effect of cetyltrimethylammonium bromide. This was explained by increased binding of the ionic surfactant to the emulsan (174).

Some strains of *Bacillus subtilis* have the ability to synthesize antibiotics. One of these antibiotics is iturin, which is a peptidolipid with antifungal activity. This compound was discovered as an antibiotic but was later described as a biosurfactant in a screening for bacteria with hydrophobic cell surfaces (137, 139). It was demonstrated that this compound inhibited the growth of the producing organism, an inhibition which differs from its antifungal activities (16). The same compound affects the morphology and membrane structure of yeast cells. Iturin A passes through the cell wall and interacts not only with the cytoplasmic membrane but also with the membranes of cytoplasmic organelles (187).

In other studies, lipids and fatty acids have been found to be effective antifoulants. This has been described for the adhesion of bacteria in the oral cavity (102). Other evidence comes from a very different environment. For a marine sponge which produces an antifouling factor, the active components could be identified as a mixture of fatty acids (58). Furthermore, fatty acids have been reported as growth inhibitors of *Neisseria gonorrhoeae*, *Bacillus subtilis*, and *Salmonella typhimurium* (123). Lipids also have an effect on the adhesion of microorganisms. Precursors and degeneration products of sphingolipids were found to inhibit the interaction of *Streptococcus mitis* with buccal epithelial cells and of *Staphylococcus aureus* with nasal mucosal cells (17).

Applied Aspects of Bacterial SACs

If microbial SACs could be applied as natural antiadhesives on surfaces exposed to the aqueous environment, they would have a great advantage compared with, e.g., the very toxic tributyl tin-containing antifouling paints still used on hulls of large ships (42, 69). More recent developments further demonstrate the potential of SACs to make surfaces nonadhesive. Those nonstick coatings have been prepared by self-assembly and immobilization of reactive polymeric surfactants (167). Antifouling paints have been on the market for many years, although the active compounds which they contain are synthetic. This offers the possibility of controlling biofouling by using the mediating layer of adsorbed molecules as opposed to targeting the microbial cell itself (47). Perhaps the key to solving the problem of biofouling lies in the surface activity of antibiotics or in the antibiotic activity of biosurfactants, depending on one's research perspective. Promising candidates for biological antibiofouling applications have been found in screenings for other natural compounds with hydrophobic groups and chains (29).

It is now clear that there is a great variety of microbial SACs. It also appears that these various compounds are very likely to play roles in very different physiological processes. The numerous types of microbial SACs, as well as their production on nonhydrocarbon substrates, suggest further specific physiological roles for these compounds. The few examples reported in the literature, which indicate physiological roles for microbial SACs other than growth on hydrocarbons, are discussed below, together with an approach to the significance of microbial SACs in adhesion and deadhesion processes at interfaces.

SURFACE-ACTIVE APPROACH TO BACTERIAL ADHESION/DEADHESION

Before discussing the different aspects of the significance of microbial SACs in adhesion to and deadhesion from interfaces, a few general points should be made. The interaction of synthetic surfactants with interfaces is a topic of extensive research, and there is a large body of literature (166). These interactions are due not only to hydrophobic interactions of the hydrophobic part of the molecule but also to the hydrophilic reactions of the hydrophilic part. This hydrophilic part may be anionic, cationic, amphoteric, or nonionic. Therefore, a range of interactions is involved in the possible adsorption of charged surfactants to interfaces. Most natural interfaces do carry an overall negative or, rarely, positive charge. Thus, the ionic conditions and the pH are important parameters if interactions of ionic surfactants with interfaces are to be investigated (7, 34, 109). Some of the possible interactions of ionic and nonionic surfactants with charged interfaces are shown in Fig. 3. In addition, the molecular structure of a surfactant will influence its behavior at interfaces. This again covers the hydrophilic as well as the hydrophobic part of the molecule, as has been shown for dimeric surfactants carrying two hydrophobic chains (83). The situation becomes more complicated if in adsorption processes both surfactants and polymers are involved. This has been demonstrated by using cationic polymers and anionic surfactants in studies of adsorption to negatively charged surfaces (177). In a recent study, measurements with lipid, polylysine, and bacterial surface layers adherent to mica surfaces were made. It was shown that not only was the intrinsic property of a lipid or protein surface important but also additional factors were significant. These factors include the environment, e.g., aqueous solution or atmosphere (humidity), the existence of adhesive contact between the molecules and another surface, and, for dynamic processes, the duration and history of the interaction with the other surface (97).

All the aspects of synthetic SACs outlined above do, in principle, apply to microbial SACs. Therefore, in outlining the surface-active approach, the basic ideas and figures in this article present a more simplistic view in which the charge of microbial SACs is neglected. In describing the surface-active approach, an attempt is made to elaborate the possible theoretical locations and orientations of the biosurfactants and amphiphilic and polyphilic compounds, as well as the different types of surfaces with which they may interact. The situation in natural systems is far more complex and requires the consideration of many additional parameters. The first ideas on the general significance of bacterial SACs in the interaction of bacteria with various interfaces were described as early as 1987 (131). Subsequently, Gerson (51) also suggested that SACs were significant in the growth of microorganisms on water-insoluble substrates.

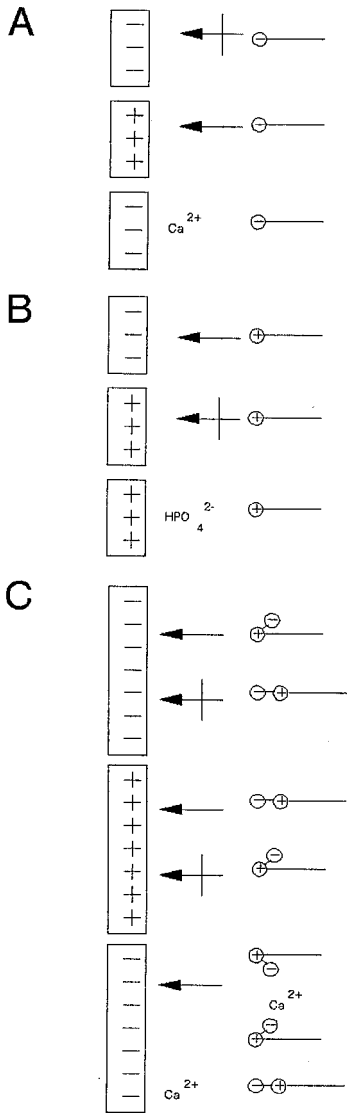

FIG. 3. Orientation of anionic (A), cationic (B), and amphoteric (C) synthetic surfactants at interfaces with negative or positive charges. The attraction of different charges by means of counterions in solution is demonstrated. The possible electrostatic attraction or repulsion is shown. The hydrophobic part of the surfactants is indicated by a straight line. Redrawn and modified from reference 109.

SIGNIFICANCE OF BACTERIAL SACs IN ADHESION TO INTERFACES

SACs Bound at the Bacterial Cell Surface

Cell-bound biosurfactants. A biosurfactant may be anchored with the hydrophobic part in the outer layers of the cell surface (the cell wall in gram-positive bacteria and the outer membrane in gram-negative bacteria). In this case, the cell can interact with a hydrophilic interface but not with a hydrophobic interface (Fig. 4A). An illustration of this case is provided by the lipids in the outer layer of the outer membrane of gram-negative cells. Depending on the cell surface structure, these lipids may be involved to a certain degree in the interaction with interfaces, although the situation at the molecular level is still unclear. Nevertheless, there are examples in which a role for biosurfactants in blocking hydrophobic sites on the cell surface was found. The reduction of cell surface hydrophobicity by the presence of a serratamolide was suggested for *Serratia marcescens* (8). Another example is the lipid-modified polypeptides in the outer layer of the cytoplasmic membrane of gram-positive bacteria. For streptococci, it could be shown that these lipid-modified polypeptides are important for adhesion, aggregation, coaggregation, and hydrophobicity (79).

The biosurfactant may also be oriented the other way around, i.e., bound via the hydrophilic part to the cell surface, thereby exposing the hydrophobic part to the outside. This would result in a hydrophobic cell surface, and in this case the cell can interact with a hydrophobic interface (Fig. 4A). The first evidence originated from pioneering work published by Dyar, who studied the cell surfaces of *Micrococcus* and *Mycobacterium* species (40). The bacteria of the genera *Corynebac-*

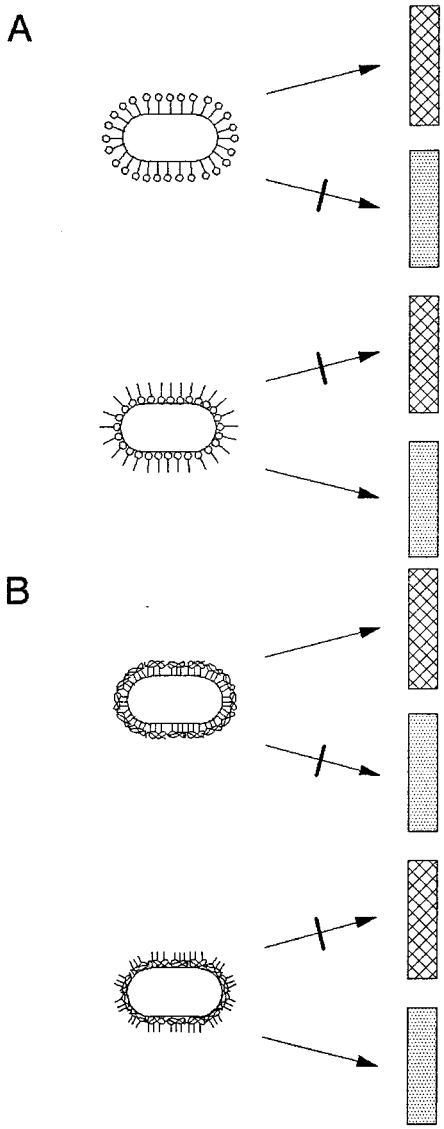

FIG. 4. Orientation of microbial biosurfactants or amphiphilic polymers (A) and polyphilic polymers (B) at the microbial cell surface. The binding of the SACs to the microbial cell surface may be mediated by hydrophobic interactions (top illustration in each panel) or ionic interactions (bottom illustration in each panel). The hydrophobic part of the surfactants is indicated by a straight line. The possible adhesion of microorganisms to interfaces with hydrophilic (hatched) or hydrophobic (dotted) properties is indicated.

terium, *Mycobacterium*, and *Nocardia* (also known as the CMN complex) have a cell wall that is rich in covalently bound hydrophobic mycolic acids. Mycolic acids are high-molecular-weight hydroxy fatty acids with a long aliphatic side chain. The number of carbon atoms in mycolic acids can vary between 30 and 90. The bacteria in this group are hydrophobic, because they have the aliphatic chain of the mycolic acids not anchored in the cytoplasmic membrane but exposed to the exterior. Coryneform bacteria with mycolic acids of various chain lengths were used to study the adhesion to Teflon and glass. It was found that physicochemical cell surface properties (hydrophobicity) and adhesion to hydrophobic Teflon could be related to the presence and chain length of mycolic acids (13). A relationship between toxin/mycolic acid production and utilization of hydrocarbons has been suggested for corynebacteria. This relationship may include a hydrophobic cell surface and the production of emulsifying compounds (181).

More evidence came from studies with *Pseudomonas aeruginosa* strains producing a rhamnolipid surfactant. It was found that the rhamnolipid increased cell hydrophobicity of slow octadecane degraders, which in turn was related to the rate of octadecane degradation. These findings imply a binding of the rhamnolipid in the outer cell layers by the hydrophilic part while the hydrophobic moiety is directed into the environment (212).

When *E. coli* is infected with *Bdellovibrio bacteriovorus*, long-chain fatty acids of the latter become covalently bound to the *E. coli* peptidoglycan during intraperiplasmic growth (188). In *E. coli*, a gram-negative bacterium, the fatty acids are not exposed to the outside. However, this illustrates that fatty acids can be bound to peptidoglycan. Nevertheless, this finding suggests that the same principle applies to gram-positive bacteria, in which those fatty acids may then be exposed to the exterior. There is an interesting report comparing the lipid content of free-living and adherent bacteria. This study showed a difference in the lipid content and of the fatty acid ratio of the adherent and free-living bacteria (192). A further example of this was found in studies with yeast cells. It was shown that fatty acids are the hydrophobic determinants on the cell surface of *Saccharomyces* strains. This implies an outside exposition of the acyl chains of the fatty acids (73, 74). In another system, a bacterial SAC has been related to specific interactions of bacteria with higher organisms. *Rhizobium leguminosarum* produces a lipooligosaccharide which was shown to be responsible for a signal which mediates host specificity with leguminous plants (179).

Cell-bound polymeric SACs. Polymeric amphiphilic molecules may also be anchored with the hydrophobic part in the outer cell layers, thereby exposing the hydrophilic region to the environment. This arrangement allows the cell to interact with hydrophilic interfaces but not with hydrophobic ones (Fig. 4B). The well-known examples of this situation are gram-negative bacteria with lipopolysaccharides (108, 153, 198) and enterobacterial common antigens (93). Other related examples include gram-positive bacteria with lipoteichoic acid (88), lipomannan (103, 147), *Actinomyces* amphiphile (201), and some other amphiphiles (77, 152, 206). In addition, gram-negative bacteria may possess polysaccharides anchored with their lipid part in the outer membrane (49, 59, 94, 95, 168). The same might be true for gram-positive bacteria. For example, a lipid membrane anchor has been found for the D-arabino-D-mannan and D-mannans of *Mycobacterium tuberculosis* (205). Apart from the lipopolysaccharide type of compounds, there is a second important group of cell surface amphiphiles which are proteinaceous. Lipoproteins are anchored via their lipid moiety in the outer layer of the outer membrane of gram-negative bacteria but also in the outer layer of the cytoplasmic membrane of gram-positive bacteria. This indicates an involvement of the lipid moiety in the interaction with interfaces (149).

The amphiphilic polymers may also be bound via the hydrophilic part to the cell surface and have the hydrophobic part directed away from the cell surface. In this situation, it would be possible for the cell to adsorb to hydrophobic interfaces and not to hydrophilic ones (Fig. 4B). The best-known examples here are lipoteichoic acids which form molecular complexes with a surface protein of streptococci (141). This protein may be identical to the M protein, although an M protein-independent mechanism has been suggested (26). The lipid moiety of lipoteichoic acids has been found to be responsible for the cell surface hydrophobicity of group A streptococci (124). It has been shown that human plasma fibronectin contains fatty acid-binding sites to which the lipoteichoic acids bind via the lipid moiety (32). On the basis of these data, Beachey (10) suggested a mechanism for the interaction of streptococci with host cells. This mechanism was supported by a recent review on streptococcal adhesins, in which a two-step model was presented. The model includes a reversible first step via hydrophobic components such as lipoteichoic acids and an irreversible second step (63).

SACs Bound at the Substratum

Excreted biosurfactants. Microorganisms excrete fatty acids, lipids, and biosurfactants into the surrounding media. A microbial cell able to excrete biosurfactants into the aqueous phase may be responsible for a microbially created conditioning film at an interface. On a hydrophobic interface, this conditioning film will change the interface from hydrophobic to hydrophilic. This means that hydrophilic cells but not hydrophobic cells may now interact with the interface (Fig. 5A).

The first example was reported for *Thiobacillus* species, which produce a SAC. This compound may be involved in the initial stages of adhesion to the hydrophobic surface of elemental sulfur (11, 20, 80). A further example is represented by microorganisms producing SACs which have been isolated from certain habitats on the basis of the presence of hydrophobic interfaces. It was reported that most phytopathogenic corynebacteria isolated from the hydrophobic cuticle of plants produce SACs (3). Similar findings were reported for *Pseudomonas* species (22).

Microorganisms producing SACs have even been isolated from aqueous habitats on the basis of their hydrophobic cell surfaces (137). It is interesting that some of the biosurfactants found during this screening were initially discovered as antibiotics (139). One such isolated bacterium was a *Pseudomonas* strain producing viscosin, a peptidolipid biosurfactant which lowers the surface tension of water to the lowest theoretical possible value of 27 mN/m (134). This viscosin was again described in a different study in which the head rot disease of broccoli was investigated. The *Pseudomonas fluorescens* strains causing the symptoms produced viscosin, which allowed the wetting of the extremely hydrophobic, waxy surface of broccoli leaves. The pectolytic and surfactant-positive strains were able to cause water-soaked areas and spreading of the decay which eventually led to the disease (65, 96). Viscosin-producing *P. fluorescens* strains were also found in a screening program for microorganisms able to grow on coal. However, no relationship between liquefication and surfactant production could be established (43, 44). Phytopathogen antagonistic bacteria were also studied for their ability to adhere to hydrophobic fruit surfaces. *Bacillus subtilis* was examined for physicochemical cell surface parameters to select strains for treatment of to-

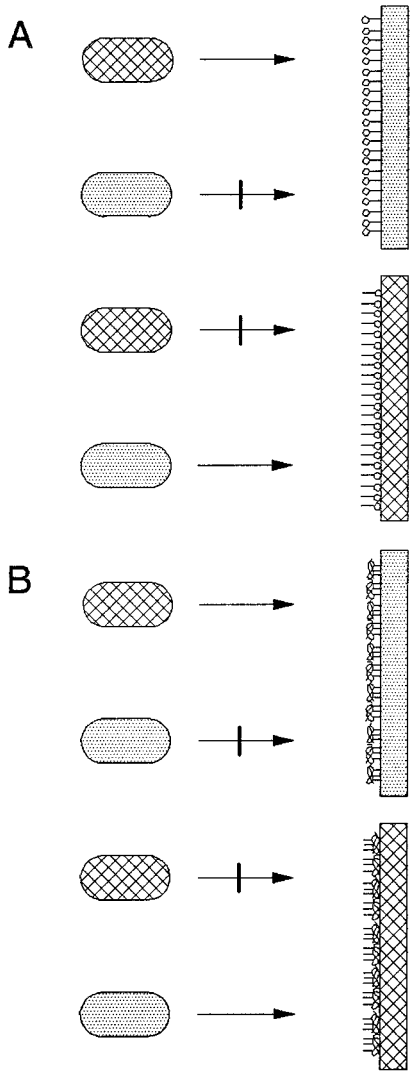

FIG. 5. Adhesion of hydrophilic and hydrophobic microorganisms to a hydrophilic (hatched) and hydrophobic (dotted) interface. The interface is covered with a microbial conditioning film of biosurfactants or amphiphilic polymers (A) and polyphilic polymers (B). Depending on the surface energy of the interface, the conditioning film of microbial SACs will have a different orientation. The microbial SACs may be bound to the interface by means of hydrophobic interactions or ionic interactions. The hydrophobic part of the surfactants is indicated by a straight line.

mato surfaces (143). However, so far no results of the role of lipopeptide production by the bacteria have been published.

Another example of excreted biosurfactants may be the difference in hydrophobicity found in a colony of a marine *Vibrio* species. The hydrophobicity increased from the periphery to the center of the colony. This colony hydrophobicity did not correlate with the cell surface hydrophobicity of the cells from the different growth regions. However, it could be correlated with the presence of a loosely bound emulsifying agent (164). This observation would mean that the hydrophobic regions of an excreted SAC are interacting with the hydrophobic air interface whereas its hydrophilic regions are interacting with the hydrophilic cell surfaces of the bacterial colony. Similar findings have been reported for *E. coli* colonies. By using electron microscopy techniques, it has been shown that agar colonies are covered by a surface film of lipid material (186). The biological role of this film is still unknown. These examples indicate a relation between microbial cell surface hydrophobicity and the production of SACs, which is discussed later.

On a hydrophilic interface, the excreted biosurfactants may change the properties of the interface from hydrophilic to hydrophobic. Only hydrophobic cells are able to interact with this hydrophobic conditioning film (Fig. 5A). An example of this is the surface-active exolipid of *Serratia marcescens*. This lipopeptide was shown to promote the flagellum-independent spreading of the bacteria on a hydrophilic surface (118, 119). These results could be verified by using spreading-defective mutants, which when serrawettin was added, were able to spread on low-agar medium (116). Similar findings have been reported for *Serratia rubidaea* (117).

Excreted polymeric SACs. Amphiphilic and polyphilic polymers may also be excreted into the environment and form a conditioning film on an interface. On a hydrophobic interface, the polymers will bind with the hydrophobic lipid part or the hydrophobic groups at the interface, thereby changing the hydrophobic interface into a hydrophilic one. Hydrophilic microorganisms, but not hydrophobic microorganisms, would now be able to interact with the hydrophilic conditioning film of microbial origin (Fig. 5B). Such behavior was reported for *Acinetobacter calcoaceticus*, which produces a polyphilic polymer called emulsan. This polymer effectively inhibited the adhesion of hydrophobic bacteria such as *Acinetobacter calcoaceticus* and *Streptococcus pyogenes* to hydrocarbons as well as to epithelial cells. It was pointed out that the polyphilic polymer interacts with the hydrophobic hydrocarbon and the hydrophobic binding sites of epithelial cells via its fatty acids (156).

In the as yet theoretical case of microbial amphiphilic polymers interacting with a hydrophilic interface, the hydrophilic interface would become hydrophobic. As a result, only hydrophobic microorganisms may be able to interact with the hydrophobic conditioning film (Fig. 5B). No such case has been found.

SIGNIFICANCE OF BACTERIAL SACs IN DEADHESION FROM INTERFACES

Biosurfactants

As described above, biosurfactants may be oriented in different ways at the microbial cell surface. However, regardless of their orientation, if they are released from the cell surface or excreted into the area between the cell surface and interface, they will probably lead to deadhesion of the bacterium from the interface. Depending on the hydrophilic or hydrophobic properties of the interface, the bacteria will leave a microbial conditioning film with hydrophilic or hydrophobic properties (Fig. 6A). For these microbial molecules left on a surface after deadhesion, the term "desorption footprints" was suggested (132). Such footprint material left on surfaces has been labelled by using lectins to identify its properties (136).

Several examples which fit into this category were described for streptococci. By using image analysis in flow cell studies, it has been found that streptococci adhere to a surface but will later deadhere by leaving behind some compounds which may influence the adhesion of other microorganisms. It was shown for *Streptococcus mitis* cells that these substances modified the substratum and influenced the adhesion of *Streptococcus mitis* and *Streptococcus mutans* cells. However, the chemical identity of the SACs is still not known (148). The same result has been found with other oral microorganisms. Surfaces preconditioned with detached *Streptococcus cricetus* cells negatively influenced the adhesion of *Prevotella intermedia* (33). In other

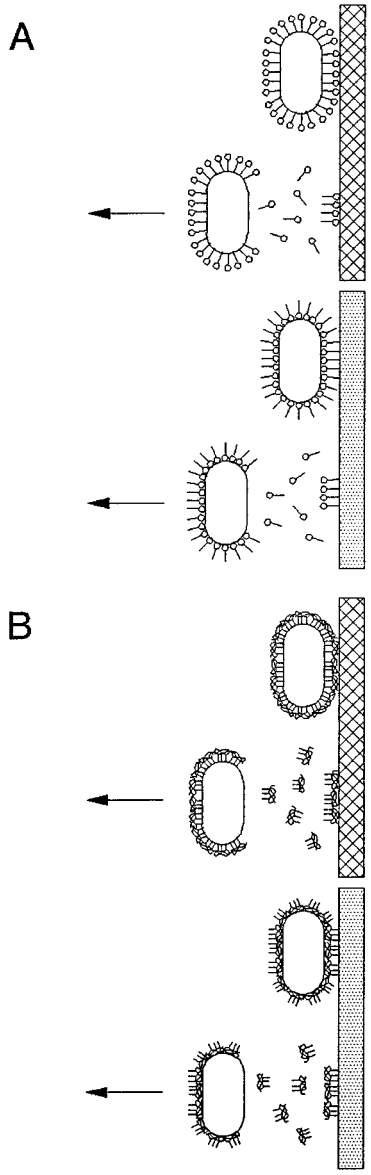

FIG. 6. Deadhesion of microorganisms from hydrophilic (hatched) and hydrophobic (dotted) interfaces by excretion or release of surface-bound microbial SACs. The SACs will result in a microbially produced footprint or conditioning film consisting of biosurfactants or amphiphilic polymers (A) and polyphilic polymers (B). The orientation of the SACs which form the footprint or conditioning film is determined by the surface energy of the interface. The microbial footprint or conditioning film may later influence the interaction of other bacteria with this interface. The hydrophobic part of the surfactants is indicated by a straight line.

experiments with *Streptococcus thermophilus*, similar observations were made (24, 25). It was proposed for all examples that during deadhesion, the initially deposited cells leave a bacterial SAC on the substratum, thereby conditioning it with an antiadhesive layer.

Polymeric SACs

Amphiphilic polymers and polyphilic polymers may be anchored with their hydrophilic or hydrophobic groups within the bacterial outer layers, thereby exposing the other regions to the outside. Microorganisms adherent to a hydrophilic or hydrophobic interface would be able to detach by releasing the polymers or parts of them from their cell surface. By such a mechanism, the bacteria could detach and leave the hydrophilic or hydrophobic interface modified by a hydrophobic or hydrophilic polymeric conditioning film (Fig. 6B). This precise circumstance has not yet been described. Nevertheless, there is a well-documented example in which such a polyphilic polymer, the emulsan of *Acinetobacter calcoaceticus* RAG-1, is involved in the desorption from hydrophobic interfaces. In this case, however, the adhesion to the hydrocarbon interface is not mediated by the polymer but via thin fimbriae (158). Emulsan, a polysaccharide substituted with fatty acids along the entire molecule (12, 176), is accumulated at the cell surface as a minicapsule when the bacteria grow on hydrocarbons (145). The emulsan molecules are released from the cell surface by an exocellular esterase (173) when the bacteria become starved (57). Rosenberg and Kaplan (157) suggested a role for emulsan as an antiadhesion factor for hydrophobic interfaces. Furthermore, they postulated that the released emulsan forms a film on the hydrocarbon droplet, thereby deadhering the cells and at the same time labeling the hydrocarbon droplet as being used. The same model has been used as one of the examples to elaborate the term "microbial footprints" and to emphasize the general ability of microorganisms to label interfaces. In this respect, the emulsan molecules have been discussed as desorption footprints (132) (see above).

GLIDING—A CONTINUOUS DEADHESION WITHIN TWO DIMENSIONS

The heterogeneous group of gliding bacteria represents a special case. Gliding may be regarded as a continuous desorption process during which the bacteria stay within a two-dimensional system. For *Cytophaga johnsonae*, it was shown that sulfonolipids and ornithine lipids are molecular determinants of gliding motility (2), that their synthesis is surface induced (1), and that they are localized in the outer membrane of the cells (53, 146). Other findings support an additional involvement of lipopolysaccharide in gliding motility (52, 54). Biosurfactants are also thought to be responsible for the driving force in some gliding bacteria. A model based on surface tension gradients for the gliding motility of *Myxococcus xanthus* was constructed by using theoretical calculations (84) as well as experimental observations (39). It was suggested that the polar excretion of a surfactant is capable of moving the cells across an interface. The involvement of hydrophobic components in the gliding process is further underlined by a study in which the screening for nonadherent and nonhydrophobic mutants selected for nonspreading mutants at the same time (204).

In summary, lipids, biosurfactants and lipopolysaccharides are components which may in some way be responsible for or involved in the gliding process of this heterogeneous group of bacteria. In other words, these microbial SACs facilitate a continuous deadhesion process. This does not represent a complete explanation for the gliding mechanism; however, as surface-active molecules, they are likely to play an important role in the gliding movement of these bacteria. Several motility mechanisms within the gliding bacteria will probably be found, some of which will include microbial SACs.

BACTERIAL CELL SURFACE HYDROPHOBICITY—REGULATION VIA BACTERIAL SACs?

As discussed above, microbial SACs may be bound to the microbial cell surface, exposing their hydrophobic part to the

outside. The controlled release of such surface compounds would result in a change of hydrophilic/hydrophobic surface properties. As a consequence, these compounds could be regarded as determinants which are responsible for the regulation of microbial cell surface hydrophobicity. It is well known that microorganisms are able to change their cell surface hydrophobicity in different growth phases, under different growth conditions, and during morphogenesis and differentiation (37, 159). In addition to the examples given in previous sections, there are other reports containing evidence for this statement. It was found that adherent bacteria deadhere themselves from biofilms by changing their cell surface hydrophobicity. Studies with *Pseudomonas aeruginosa* and *E. coli* revealed a higher hydrophilicity for the cells which were dispersed from a biofilm (4, 5).

Several microbial compounds have been suggested to be hydrophobic cell surface determinants (hydrophobins). These include thin fimbriae, M protein/lipoteichoic acid, A protein, protein layer, prodigiosin, glucosyltransferase, outer membrane proteins, surface fibrils, various fimbriae, core oligosaccharides/outer membrane lipids, and gramicidin S (159). However, apart from lipoteichoic acid, this list does not include microbial SACs in general. Furthermore, the role of lipids in the outer layer of the outer membrane in cell surface hydrophobicity has not been explained. It is known that gram-negative bacteria with a reduced O-specific chain in their lipopolysaccharide appear to be less hydrophilic than strains having the full O-specific chain. However, the function of outer membrane lipids in this respect is not fully understood.

Microbial SACs would be ideal for effecting a certain favorable cell surface hydrophobicity or hydrophilicity, depending on the orientation at the cell surface. The binding of biosurfactants and amphiphilic polymers to the microbial cell surface, combined with a release mechanism, suggests an ideal way for microorganisms to regulate the cell surface hydrophobicity and thereby adjust to changing environmental conditions.

CONCLUSIONS

The evidence from published research, taken together with the suggested surface-active approach, confirms that there is a significant role for microbial SACs in adhesion to and deadhesion from interfaces. The heretofore generally accepted natural function of microbial SACs in bacterial growth on water-insoluble substrates such as hydrocarbons seems to be only a special case. However, SACs produced by microorganisms are ideally suited to mediate the interaction—adhesion and deadhesion—between microorganisms and interfaces. Furthermore, SACs may play a role in the movement of gliding bacteria across interfaces and the regulation of bacterial cell surface hydrophobicity. In addition, microbial SACs may be among the candidates for chemical communication between bacteria if they influence the interaction of other bacteria with interfaces.

Most interesting in terms of evolutionary aspects is the theory of surface metabolism. It has been proposed that life on Earth began via two-dimensional "surface organisms." According to this theory, surface-bound SACs produced by the surface organisms had a key function in surface metabolism and finally in three-dimensional cellular organization (195).

Because of the overall significance of bacterial adhesion and deadhesion both natural and technical systems, it is necessary to further study the molecular basis of these interactions. Future research in the fields of microbial cell surface structure, microbial interaction with interfaces, and chemical messengers of bacteria will help to unequivocally establish the role of microbial SACs as mediators between bacteria and interfaces, regardless of whether biosurfactants, amphiphilic polymers, or polyphilic polymers are involved.

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