The effect of adhesion on survival and growth of microorganisms

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Summary. Adhesion of microorganisms to solid surfaces or water/air interfaces can significantly influence cellular metabolic activity, development and viability. Attachment is of advantage particularly for organisms growing under oligotrophic or otherwise extreme conditions. However, the ability to detach and migrate is of vital importance when prevailing conditions become too harsh or in situations of population explosion.
Adhesion can cause alterations in the physical and chemical properties of substratum surfaces as well, by means of degradation, aggregation, emulsification etc.
Key words. Adhesion; metabolic activity; detachment; interfaces.

Introduction

Adhesion of microorganisms to surfaces is of profound importance for degradation or transformation of solutes and water-immiscible substrates as well as for modification of surface properties or even corrosion of solids. For example, modern treatment of waste-water depends on the development of a biofilm on some solid support for uptake and degradation of soluble organic or nitrogenous compounds [30], and microbial decomposition of cellulose fibers requires prior attachment of cellulolytic bacteria to this substrate [45].
The physiology and even viability of microorganisms can be strongly affected by adhesion to solid surfaces or air/water interfaces. Rational treatment of solids, solutes or water-immiscible liquids therefore depends on understanding the implications of adhesion for survival and growth, and the circumstances under which detachment takes place.

Effects of adhesion on survival, growth and metabolic activity

The mechanisms which facilitate adhesion can be specific for the colonized surface, e.g. lectin-sugar interaction, or non-specific, such as hydrophobic interactions, but subsequent growth also depends on other physico-chemical parameters (pH, temperature, etc.) and availability of suitable nutrients. The combination of all these factors allows growth of only certain species even when non-specific adhesion is involved.
While specific recognition mechanisms are important for adhesion to nutrient substrates (adhesion of pathogenic or parasitic microorganisms to living tissues is outside the scope of this review), 'non-specific' mechanisms facilitate adhesion to both nutrient and 'inert' surfaces.

Nutrient substrates

Many economically and environmentally important processes, in which various microorganisms degrade and metabolize solid or water-insoluble substrates, are dependent on adhesion of the cells to their substrate surfaces. Adhesion probably minimizes dissipation and waste of the extracellular enzymes which are usually involved in these processes. A few examples will illustrate this situation:
Clostridium thermocellum cells are attached to cellulose fibers, which serve as their substrate, during the early and logarithmic growth phases [45]. When the medium pH drops to unfavorably low values the cells undergo complete sporulation while still attached to the fibers. The resistant spores thus remain in the vicinity of their future substrate until environmental conditions improve and facilitate germination.
The hydrocarbon-utilizing *Acinetobacter calcoaceticus* cells attach to droplets of crude oil through their hydrophobic fimbriae [41]. A hydrophilic mutant, which could not attach at the oil/water interphase, failed to grow on the hydrocarbon substrate.
Thiobacillus sp., which can oxidize pyrite to ferrous ions and elemental sulfur, was shown to attach preferentially to pyrite particles in pyrite coal [43]. Adsorption of the cells to the mineral substrate is thought to be the rate-limiting step in the leaching process of such coal, which provides energy for these chemoautotrophic bacteria.
The specific attachment of *Rhizobium* cells to roots of leguminous plants via lectin-sugar interactions [2] is a prerequisite for the symbiosis maintained by this plant-microbe partnership. The bacteria are attracted by and utilize root exudates, and then colonize the roots to form nodules which fix atmospheric nitrogen and make it available to the plant.

Inert surfaces: the terrestrial environment

Mobility in soil ecosystems is obviously much more restricted than in water-bodies or in the air. Nevertheless, terrestrial microorganisms are not necessarily immobilized. Rather, they can move through the interstitial pore water or gas (depending on the state of saturation of the soil) or directly over adjacent particles. It is therefore of interest to examine cases in which microbial cells are firmly attached to soil components, and to ponder on the possible benefits they may derive from such interactions.

Filamentous cyanobacteria play a major role in stabilization of exposed soils[4]. A *Microcoleus* sp. was found to stabilize moving sand dunes through its mucilagenous sheath, which acts as a cementing agent[46]. This and other filamentous species can form a continuous mat which traps and immobilizes the underlying sand particles (Y. Bar-Or and A. Danin, unpublished results). Fungi and mosses are also involved in this process, either through extracellular excretions or by mechanical binding of the soil particles. The ability of the soil cyanobacteria to attach to the upper, erosible layer of soil is the first step in the establishment of a stable population. Later drying of the mats leads to the formation of typical crusts, which are resistant to prevalent conditions of low water potential[6], high temperatures[8], and intense solar radiation.

Soil microorganisms, including bacteria, cyanobacteria, fungi and algae, contribute significantly to soil aggregation through excretion of extracellular polymeric substances which flocculate soil particles[33] and through adsorption and bridging of adjacent particles by the cells[29]. This leads to improved aeration and percolation of water through the soil matrix, creating better conditions for further microbial growth along the soil profile.

Adsorption of microbial cells to soil particles[13, 44] as well as other solid surfaces[47] is known to enhance the activity of a large number of enzymes, and particle-bound cells were found to be more active in uptake of glucose and glutamate than freely suspended cells[23]. The mechanisms of activation are not clear, but may include protein structure modification or alteration of membrane properties of the cells, which influence diffusion and transport of solutes.

Adhesion sometimes affects microbial metabolism in more subtle ways; particles of the clay mineral montmorillonite attached to the hyphae of the pathogenic fungus *Histoplasma capsulatum* can cause a reduction in respiration[26], and this has been proposed as an explanation for the absence of this organism from montmorillonite-containing soils.

Inert surfaces: the aquatic environment

Microorganisms in aquatic ecosystems are found either along the water column or attached to various submerged objects, to the sediment, or at the air/water interface.

Biofilms, which are multilayers of microbial cells adhering to submerged objects or to walls of pipes, tunnels and other engineered passages for water[9], have important effects on various industrial operations. In waste-water treatment they remove organic and nitrogenous compounds. In heat-exchangers of power stations etc. their presence causes a large decrease in the heat conductivity, and hence in the effectivity, of the process. Accumulation of microbial cells is also found at interfaces between water and air and water and benthos; in the ocean, the population density found at the surface[3, 35] is usually several orders of magnitude higher than in the bulk water. The movement of cells out of the bulk water indicates that adhesion may offer several advantages for growth, including: 1) A continuous supply of nutrients flowing over the immobile cells. 2) Continuous removal of (toxic) metabolic waste products. 3) Higher nutrient levels, since various organic molecules tend to concentrate at water/air interfaces[12]. Thus copiotrophic bacteria, which require relatively high concentrations of nutrients, can grow even in water-bodies where nutrients are otherwise dilute, when adhering to such interfaces[20].

Nutrient availability may, however, be too simplistic an explanation for the high cell numbers routinely encountered at surface microlayers, since contradictory results have been reported for metabolic activities of attached cells as compared to free-living cells. While attached cells were often shown to exhibit enhanced activity[13, 15, 44], several studies revealed that metabolic activities of interface-associated bacteria were not always higher than those of cells sampled from the bulk water[5, 14, 18]. Bulk water was found sometimes to have a higher capacity for hydrocarbon degradation than the surface microlayer although it contained higher concentrations of nutrients (including hydrocarbon, phosphate and organic matter) and viable cells than the bulk water[16]. This discrepancy need not be surprising since accumulation of cells at the surface is caused, at least in part, by various physico-chemical factors (e.g. cell surface hydrophobicity[11], sweeping by bubbles[3] etc.) which do not necessarily select for cells which are particularly adapted to the nutrient composition and levels found at surfaces. Furthermore, activity is usually measured as the capacity of the test organisms to metabolize various molecules, and elevated activity can be expected only when both cells and substrate adsorb to the surfaces under investigation and when the substrate remains utilizable even after being adsorbed. Attached and non-attached cells may even belong to different species which have dissimilar physiological properties, which makes comparison between them irrelevant. This has been illustrated in a study on bacterial accumulation at model lipid surface films[36]. It was found that the lipid layer was ten times more enriched in a *Serratia* sp. than in *Aeromonas* and *Pseudomonas* spp., and a wild-type *Serratia* sp. was able to scavenge fatty acids sorbed on glass much more effectively than a relatively hydrophilic mutant strain[22]. Perhaps more attention should be given to the metabolic activities of surface-bound cells isolated directly from natural ecosystems, since only such cells can be presumed with certainty to derive some benefit from adhesion.

Physiological comparison of attached and non-attached populations is made even more complicated because of the dynamic equilibrium maintained between the two groups[32]. The finding that irreversible adsorption of nitrifying bacteria to various particles is accompanied by a dramatic increase in activity and that this effect is due to adhesion per se[13] is therefore of particular interest.

Attachment at the water/air interface of deep water-bodies is an obvious must for phototrophic microorganisms, such as microalgae and cyanobacteria, which require light for growth. This often leads, under suitable conditions of light, temperature and water flow, to the development of cyanobacterial scums [37] or even 'hyperscums' several decimeters thick [48].

During the night, in the absence of light, there is migration of the scum-formers from the upper, nutrient-deficient layers to the lower, nutrient-enriched layers [28,38]. This diel movement is made possible by accumulation of products of photosynthesis during the day, which increases the specific gravity of the cells and facilitates their sedimentation. Degradation of the carbohydrates during the night makes the cells able to float once again to the photic zone.

Adhesion to suspended or sedimented particulate solids is known to increase the viability of bacteria in aquatic environments. This is true both for endogenous populations, such as nitrifying bacteria [13], and for exogenous microorganisms such as those of the digestive tract [7,27], which are carried with sewage into lakes or other receiving bodies of water. Various factors may contribute to this phenomenon, e.g. increased nutrient availability, binding and neutralization of various toxic substances by the sorbing surface, and protection against degrading enzymes, phages and other microbial or zooplankton predators [39]. Another possible mechanism is reduction in shear forces operating on the bacterial cell, and the resulting better preservation of the cell wall, after adsorption on a solid surface.

Microbial mats, covering large surface areas, are found in many hot, saline, or otherwise extreme environments [10]. Their extensive dimensions are possibly due to reduced competition and predation and to the ability of the cells to adapt physiologically to prevailing conditions. Such adaptability is of value only so long as the cells remain attached at or near the extreme environment to which they are adapted. As the water flows away from its source it cools down or is diluted and the peculiar properties of the colonizing microflora then become of no significance.

Detachment – why and how

Adsorption of microorganisms to solid surfaces or to air/water interfaces is often an equilibrium situation [32]. This means that under a given set of conditions only a certain percentage of a specific microbial population is found attached, while another fraction of the same population remains unadsorbed. The equilibrium is usually a dynamic one [15,22,24], and attached cells can desorb and move to the bulk phase (and vice versa).

Changes in environmental or physiological conditions can induce a shift towards adsorption or desorption, but its direction can be different under various, apparently similar circumstances. The anaerobic, cellulose degrading *Clostridium thermocellum* adheres avidly to cellulose fibers, but cells detach when monomeric sugars are present. A drop in the medium pH leads to sporulation, and the spores remain attached to the insoluble substrate [45]. On the other hand, the aerobic spore-former *Bacillus subtilis* attaches to ion exchange resins [17], but the spores formed detach more readily than vegetative cells. It has been postulated that adhesion of the *Clostridium* spores ensures a ready source of nutrients when environmental conditions allow germination [45], while the best strategy for the *Bacillus* spores is to detach and thus to be able to be swept to some other, more hospitable environment.

In the aquatic world, increased adhesion has been proposed as a survival mechanism for starved saprophytic *Leptospira* cells [21] which can utilize fatty acids adsorbed at surfaces, while over-developed biofilms occurring on engineered pipes or walls periodically slough off and are detached [19]. It is thought that formation of anaerobic conditions, lack of nutrients and accumulation of waste by-products leads to weakening of the adhesive forces holding the biofilm together.

Attachment to surfaces via hydrophobic [40] or electrostatic [34] interactions depends on the biochemical and structural properties of the cell-surface. Detachment is therefore facilitated by modulation of surface components. It has been shown that incubation of the benthic cyanobacterium *Phormidium* J-1 in the presence of chloramphenicol (an inhibitor of protein synthesis) led to a sharp decrease in hydrophobicity, with concomitant changes in the ultrastructure of the cell-wall [1]. Masking of the cell surface is another way to alter its physico-chemical characteristics. The oil-degrading *Acinetobacter calcoaceticus* produces an amphiphilic polysaccharide which forms a capsule around the cells upon reaching the stationary growth phase. As a result the cells are rendered more hydrophilic and are thereby able to detach from the oil/water interphase [42].

Conclusions

While adhesion is essential for the development and maintenance of microbial societies in various ecosystems, it can also become a trap when environmental conditions change for the worse or when available nutrients are exhausted. The ability to detach and migrate then becomes a clear asset. The collapse of cyanobacterial scums, accompanied by decomposition and putrefaction [25], is a good example for the damage done to both the cells and the quality of the environment when there are no efficient mechanisms for detachment and dispersal of overcrowded populations.

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1 Bar-Or, Y., Kessel, M., and Shilo, M., Modulation of cell surface hydrophobicity in the benthic cyanobacterium *Phormidium* J-1. Archs Microbiol. *142* (1985) 21–27.

2 Bauer, W. D., Infection of legumes by Rhyzobia. A. Rev. Plant Physiol. *32* (1981) 407–449.
3 Blanchard, D. C., and Syzdek, M., Mechanism for the water-to-air transfer and accumulation of bacteria. Science *170* (1970) 626–628.
4 Booth, W. E., Algae as pioneers in plant succession and their importance in erosion control. Ecology *22* (1941) 38– 46.
5 Bright, J. J., and Fletcher, M., Amino acid assimilation and electron transport system activity in attached and free-living marine bacteria. Appl. envir. Microbiol. *45* (1983) 818–825.
6 Brock, T. D., Effect of water potential on a *Microcoleus* (cyanophyceae) from a desert crust. J. Phycol. *11* (1975) 316– 320.
7 Burton, G. A., Gunnison, D., and Lanza, G. R., Survival of pathogenic bacteria in various freshwater sediments. Appl. envir. Microbiol. *53* (1987) 633–638.
8 Buzer, J. S., Dohmeier, R. A., and Du Toit, D. R., The survival of algae in dry soils exposed to high temperatures for extended time periods. Phycologia *24* (1985) 249–251.
9 Characklis, W. G., and Cooksey, K. E., Biofilms and microbial fouling. Adv. appl. Microbiol. *29* (1983) 93– 138.
10 Cohen, Y., and Rosenberg, E. (Eds), Microbial Mats: Physiological Ecology of Benthic Microbial Communities. Am. Soc. Microbiol., Washington 1989.
11 Dahlback, B., Hermansson, M., Kjelleberg, S., and Norkrans, B., The hydrophobicity of bacteria – an important factor in their adhesion at the air-water interface. Archs Microbiol. *128* (1981) 267–270.
12 Davies, J. T., and Rideal, E. K., Interfacial phenomena. Academic Press, London 1963.
13 Diab, S., and Shilo, M., Effect of adhesion to particles on the survival and activity of *Nitrosomonas* sp. and *Nitrobacter* sp. Archs Microbiol. *150* (1988) 387–393.
14 Fletcher, M., A microautoradiographic study of the activity of attached and free-living bacteria. Archs Microbiol. *122* (1979) 271–274.
15 Fletcher, M., Measurement of glucose utilization by *Pseudomonas fluorescens* that are free-living and that are attached to surfaces. Appl. envir. Microbiol. *52* (1986) 672–676.
16 Guerin, W. F., Phenanthrene degradation by estuarine surface microlayer and bulk water microbial populations. Microb. Ecol. *17* (1989) 89–104.
17 Hattori, R., Growth and spore formation of *Bacillus subtilis* adsorbed on an anion-exchange resin. J. gen. appl. Microbiol. *22* (1976) 215–226.
18 Hermansson, M., and Dahlback, B., Bacterial activity at the air/water interface. Microb. Ecol. *9* (1983) 317–328.
19 Howell, J. A., and Atkinson, B., Sloughing of microbial film in trickling filters. Water Res. *10* (1976) 307–315.
20 Jones, J. G., and Simon, B. M., Nutritional strategy of a benthic filamentous bacterium. Microb. Ecol. *12* (1986) 323–330.
21 Kefford, B., Humphrey, B. A., and Marshall, K. C., Adhesion: a possible survival strategy for Leptospires under starvation conditions. Curr. Microbiol. *13* (1986) 247–250.
22 Kefford, B., Kjelleberg, S., and Marshall, K. C., Bacterial scavenging: utilization of fatty acids localized at a solid-liquid interface. Archs Microbiol. *133* (1982) 257–260.
23 Kirchman, D., and Mitchell, R., Contribution of particle-bound bacteria to total microheterotrophic activity in five ponds and two marshes. Appl. envir. Microbiol. *43* (1982) 200–209.
24 Kjelleberg, S., and Stenstrom, T. A., Lipid surface films: interaction of bacteria with free fatty acids and phospholipids at the air/water interface. J. gen. Microbiol. *116* (1980) 417–423.
25 Lanza, G. R., and Silvey, J. K. G., Interactions of reservoir microbiota: eutrophication-relates environmental problems, in: Microbial Processes in Reservoirs. Ed. D. Gunnison. Dr. W. Junk Publishers, Dordrecht 1985.
26 Lavie, S., and Stotzky, G., Adhesion of the clay minerals montmorillonite, kaolinite, and attapulgite reduces respiration of *Histoplasma capsulatum*. Appl. envir. Microbiol. *51* (1986) 65–73.
27 LeChavalier, M. W., Cawthon, C. D., and Lee, R. G., Factors promoting survival of bacteria in chlorinated water supplies. Appl. environ. Microbiol. *54* (1988) 649–654.
28 Lieberman, O., Ph. D. Thesis. The Hebrew Univ. of Jerusalem, Jerusalem 1989.
29 Lynch, J. M., and Bragg, E., Microorganisms and soil aggregate stability. Adv. Soil Sci. *2* (1985) 133–171.
30 Mack, W. N., Mack, J. P., and Ackerson, A. O., Microbial film development in a trickling filter. Microb. Ecol. *2* (1975) 215–226.
31 Marshall, K. C., Interfaces in Microbial Ecology, pp. 69–70. Harvard Univ. Press, Cambridge 1976.
32 Marshall, K. C., Mechanisms of bacterial adhesion at solid-water interfaces, in: Bacterial Adhesion, pp. 133–161. Eds D. L. Savage and M. Fletcher. Plenum Press, New York 1985.
33 Metting, B., and Rayburn, W. R., The influence of a microalgal conditioner on selected Washington soils: an empirical study. Soil Sci. Soc. Am. J. *47* (1983) 682–685.
34 Mozes, N., Marchal, F., Hermess, M. P., Van Haecht, J. L., Reuliaux, L., Leonard, A. J., and Rouxhet, P. G., Immobilization of microorganisms by adhesion: interplay of electrostatic and nonelectrostatic interactions. Biotechnol. Bioeng. *30* (1987) 439–450.
35 Norkrans, B., Surface microlayers in aquatic environments, in: Advances in Microbial Ecology, vol. 4, pp. 51–85. Ed. M. Alexander. Plenum Press, New York 1980.
36 Norkrans, B., and Sorensson, F., On the marine lipid surface microlayer-bacterial accumulation in model systems. Bot. mar. *20* (1977) 473–478.
37 Reynolds, C. S., and Walsby, A. E., Water blooms. Biol. Rev. *50* (1975) 437–481.
38 Rijn van, J., and Shilo, M., Carbohydrate fluctuations, gas vacuolation, and vertical migration of scum-forming cyanobacteria in fishponds. Limnol. Oceanogr. *30* (1985) 1219–1228.
39 Roper, M. M., and Marshall, K. C., Modification of the interaction between *Escherichia coli* and bacteriophage in saline sediment. Microb. Ecol. *1* (1974) 1–13.
40 Rosenberg, M., and Kjelleberg, S., Hydrophobic interactions: role in bacterial adhesion, in: Advances in Microbial Ecology, vol. 9, pp. 353–393. Ed. K. C. Marshall. Plenum Press, New York 1986.
41 Rosenberg, M., and Rosenberg, E., Role of adherence in growth of *Acinetobacter calcoaceticus* RAG-1 on hexadecane. J. Bact. *148* (1981) 51–57.
42 Rosenberg, E., Kaplan, N., Pines, O., Rosenberg, M., and Gutnick, D., Capsular polysaccharides interfere with adherence of *Acinetobacter calcoaceticus* to hydrocarbons. FEMS Microbiol. Lett. *17* (1983) 157–160.
43 Silverman, M. P., Mechanism of bacterial pyrite oxidation. J. Bact. *94* (1967) 1046–1051.
44 Soehngen, N. L., Einfluß von Kolloiden auf mikrobiologische Prozesse. Z. Bakt. Parasit. Infect. 2 Abt. *38* (1913) 622– 647.
45 Wiegel, J., and Dykstra, M., *Clostridium thermocellum:* adhesion and sporulation while adhered to cellulose and hermicellulose. Appl. Microbiol. Biotechnol. *20* (1984) 59–65.
46 Winder de, B., Pluis, J., de Ruis, L., and Mur, L. R., Characterization of a cyanobacterial, algal crust in the coastal dunes of the Netherlands, in: Microbial Mats: Physiological Ecology of Benthic Microbial Communities. Am. Soc. Microbiol., Washington 1989.
47 ZoBell, C. E., The effect of solid surfaces upon bacterial activity. J. Bact. *46* (1943) 39–56.
48 Zohary, T., Hyperscums of the cyanobacterium *Microcystis aeruginosa* in a hypertrophic lake. J. Plankton Res. *7* (1985) 399–409.

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