Settlement behavior of swimming algal spores on gradient surfaces

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When surfaces possessing gradients of surface energy are incubated with motile spores from the green seaweed *Ulva*, the spores attach on the hydrophilic part of the gradient in larger numbers than they do on the hydrophobic part. This result is opposite to the behavior of the spores observed on the homogeneous hydrophobic and hydrophic surfaces. The data suggest that the gradients have a direct and active influence on the spores, which may be due to the biased migration of the spores during the initial stages of surface sensing. © *2006 American Vacuum Society.*
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I. INTRODUCTION

The green seaweed *Ulva* (syn. *Enteromorpha*[1]) is one of the most common organisms that abundantly colonize various surfaces in seawater environments. *Ulva* produces motile, quadriflagellate, naked spores (zoospores), the body of which is 7–10 μm long. Spores settle on a solid substrate through a process that involves surface "sensing" and temporary adhesion[2] followed by discharge of a hydrophilic, glycoprotein adhesive[3–5] to form a permanent attachment. If the substrate is suboptimal for settlement, the zoospores swim away after initial sensing and/or temporary attachment to explore more hospitable substrates. Many factors influence the attachment of these zoospores to solid substrates, including surface polarity, energetics,[6,7] topography[8,9] as well as the chemotactic signals received from microbial biofilms.[10,11] Previous studies using self-assembled monolayers (SAMs) of alkyl thiols terminated with methyl (CH_3) and hydroxyl (OH) groups as well as their mixtures showed that the number of spores that adhered (settled) was positively correlated with hydrophobicity. In particular, the number of cells adhering to a methyl surface ($\theta_{AW}=110°$) was almost ten times as high as those adhering to a hydroxyl surface ($\theta_{AW}=20°$).[6] Swimming spores also preferentially congregate above a hydrophobic sector compared to an adjacent hydrophilic sector.[6]

These previous observations prompt the question: what happens when swimming spores are challenged with a surface possessing a continuous gradient of hydrophobicity? This question is partly related to a much older observation of Carter[12] that motile cells exhibit haptotactic movements on a gradient surface. The question is also motivated by the observations[13–15] that surface energy gradients propel liquid drops toward the region of higher wettability. In the absence of a diffusive chemotactic signal that biases the movement of the motile cells towards chemoattractants, could swimming zoospores sense the gradient? If so, would the pattern of settlement of adhered zoospores reflect the underlying gradient of surface energy? To address these questions, we assessed the attachment behavior of *Ulva* zoospores on glass slides possessing radially inward and outward gradients of surface energy. In this communication, we report that the wettability gradients disrupt the normal pattern of attachment of the swimming zoospores dramatically.

II. MATERIALS AND METHODS

A. Surface preparation

The test surfaces for this study had radially outward or inward gradients of surface energy prepared by diffusion-controlled silanization of glass slides. Fisher brand precleaned plain microscope slides (3×1 in.) were first soaked in piranha solution (mixture of 30% H_2O_2 (50% v/v solution) and 70% v/v H_2SO_4) for 30 min, then rinsed with copious amounts of distilled water. The slides were dried with ultrahigh purity nitrogen gas and subjected to oxygen plasma at 0.2 Torr for 45 s on the lowest setting in a Harrick Plasma Cleaner (Model PDC-32G) immediately before the preparation of the gradient. To prepare the outward gradient (hydrophobic center, hydrophilic periphery), a small drop (~1 μl) of dodecyltrichlorosilane [$Cl_3Si(CH_2)_{11}CH_3$] was suspended about 1 mm above the center of a clean glass slide for a total adsorption time of 12 min. The silane evaporated from the drop and diffused radially while reacting with the glass slide. The central part of the slide, closest to the drop, became maximally hydrophobic, with the contact angle of water $\sim100°$, whereas its peripheral zone remained wettable by water. Inward gradients (hydrophobic periphery, hydrophilic center) were prepared by suspending a silane-saturated filter paper, which had a circular hole of 12 mm diameter, at a distance of 1 mm from the clean glass slide for a total adsorption time of 1.5 min. The filter paper was tautly stretched across a rigid frame to prevent any buckling during the adsorption. In this reversed gradient, the part of the glass slide closest to the center of the hole was hydrophilic, but its surrounding zone became hydrophobic. Further details about the requirement of humidity and adsorption conditions can

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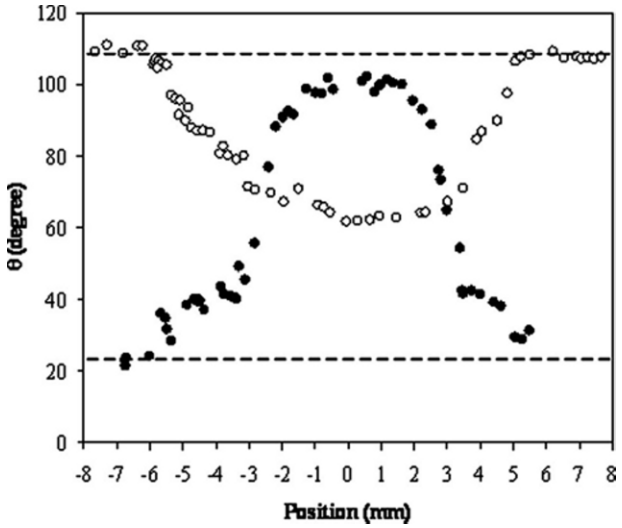

FIG. 1. Contact angles of water on outward (●) and inward (○) gradients of dodecyltrichlorosilane on glass. The dashed lines correspond to the background wettabilities.

be found in Daniel *et al.*[14] The typical wettability behaviors of the two types of surfaces are shown in Fig. 1. Along with these gradient surfaces, a set of hydrophilic slides cleaned by piranha solution and oxygen plasma as well as a set having a homogeneous hydrophobicity, prepared by vapor phase adsorption of dedodecyltrichlorosilane ($Cl_3Si(CH_2)_{11}CH_3$), were used as controls. All the surfaces were prepared at Lehigh University and sent to the University of Birmingham, UK, for the spore settlement studies via 24 h delivery. In order to prevent contamination, the slides were immersed in de-ionized/distilled water in tightly sealed glass Coplin jars before shipment. The spore settlement studies were usually done within two days after receiving the samples.

B. Spore settlement studies

Zoospores were released into Tropic Marine artificial seawater (ASW) (35.5 g/l), *p*H 8.1 as described in Callow *et al.*[2] Ten ml of a suspension of freshly released spores (1×10^6 ml^{-1}) were added to compartments of a Quadriperm dish (Greiner) each containing a slide. After incubation for 1 h in the dark, the slides were rinsed in ASW to remove nonadhered spores, fixed with 2% glutaraldehyde (v/v) in ASW, washed in 50% ASW:distilled water (DW), then DW and dried. The number of spores was counted in an area 240×180 μm and data expressed as adhered spores per 0.25 mm^2. Spores were counted every 0.5 mm across the diameter of each gradient using image analysis as described previously.[8] On uniformly hydrophilic and hydrophobic slides, the mean number of spores adhered was obtained from 30 counts on each of three replicates (x=90).

III. RESULTS AND DISCUSSION

The areal density of spores adhered to the uniformly hydrophilic and hydrophobic surfaces were 126±9 and 230±14/0.25 mm^2, respectively (arrowed on Fig. 2). The

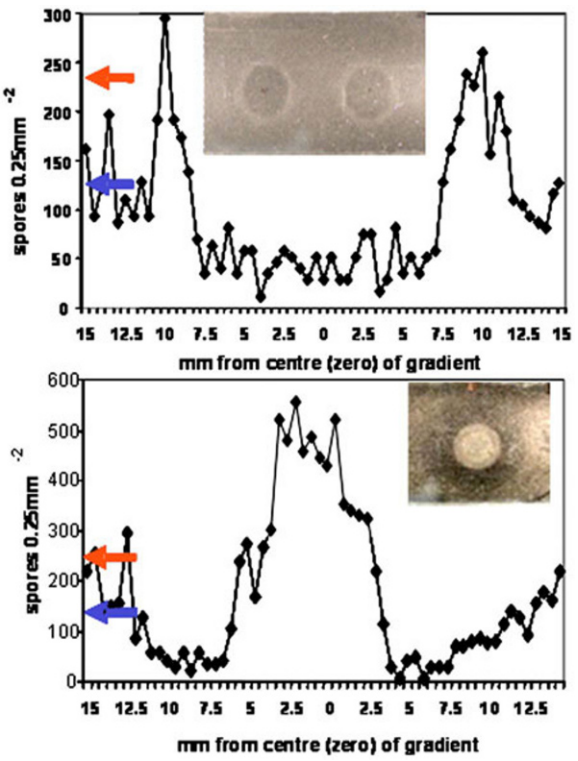

FIG. 2. Density of adhered spores across a 5 mm radius outward (Fig. 2/top) and a 5 mm radius inward (Fig. 2/bottom) radial gradient. The arrows indicate the spore density on the uniformly hydrophobic (top arrow) and uniformly hydrophilic (bottom arrow) surface. The insets show images of the gradients. The highest density of spores corresponds to the lighter regions.

higher number of spores adhered to the hydrophobic background compared to that of the hydrophilic surface concurs with data obtained for a range of surface types including SAMs.[6,7] However, the trend towards enhanced attachment on hydrophobic surfaces is reversed on the gradients. Figure 2 (top) shows that spore attachment is uniformly low across, and several mm beyond the circumference of a 5 mm radius outward gradient while a band of enhanced spore attachment is seen at a radius of approximately 9–10 mm from the center of the gradient.

The density of spores attached to the hydrophilic background reached a value similar to that on the uniformly hydrophilic surface (lower arrow) 12–15 mm from the center of the gradient. Some variation in spore numbers is seen within the area influenced by the gradient but the trend was seen consistently for replicate gradients within one experiment and between four separate experiments. The effect was reversed when the spores were incubated with inward gradients. Figure 2 (bottom) shows high numbers of spores attached within the area of the gradient, spore density being approximately double that on the uniformly hydrophobic surface as indicated by the upper arrow on Fig. 2(b). Moreover, maximum attached spore numbers are associated with the most hydrophilic central region of the gradient. The spore attachment density is lowest outside the periphery of the applied gradient, while the density of spores on the hydropho-

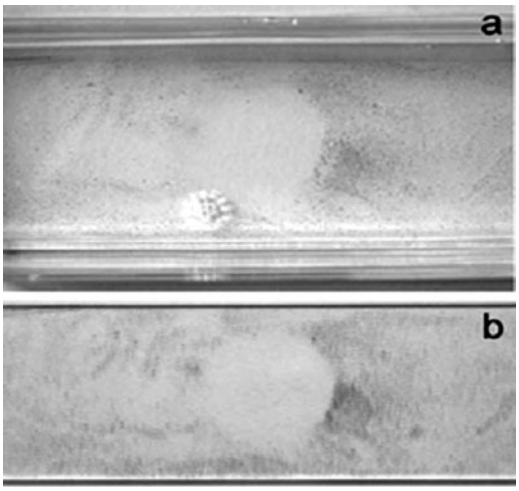

FIG. 3. Distribution of zoospores on a microscope slide with an outward gradient surface. Figure 3(a) shows the slide immediately after removal into light and while still immersed with the spore suspension (the edges of the dish and the manufacturer's mark can be seen on this image). Here, the darker areas represent both swimming and settled spores. Figure 3(b) shows the pattern on the same slide after the unsettled swimming spores were removed from the dish by gradual dilution with fresh seawater (the slide was removed from the dish to photograph).

bic background, 11–12 mm from the center of the gradient, is similar to that on the uniformly hydrophobic samples (lower arrow in Fig. 2 top).

It has been observed during the removal stage of the gradient slides from seawater that the water film occasionally dewets rapidly on the outward gradient surface before the slide could be subjected to rinsing by fresh seawater. One consequence of this rapid film breakup might be that adhered cells are swept from the central region of the gradient, which is most hydrophobic. This and the possible evaporation/convection driven liquid transport near the contact line could lead to a net accumulation of the adhered spores in the outer region of the gradient thus resulting in a spore deposition pattern resembling the current observation. In order to ensure that the results reported in Fig. 2 were not the results of the types of redistribution artifacts mentioned above, we also examined the spore settlement pattern while the slides were still immersed in seawater, i.e., immediately following the 1 h settlement period in darkness, and then after removing the nonattached spores by gradual dilution of the suspension by fresh seawater. The general pattern of the observations that the spores had avoided settling in the gradient zone was also evident in the above experiment as shown in Fig. 3.

The data suggest that the gradients have a direct and active influence on the spores since the pattern of settlement is opposite to that predicted in terms of wettability, and the area of influence is almost twice that of the applied gradient. In order to discuss the forces that might be responsible for the observed patterns of adhered spores, we need to consider aspects of the two key stages involved in spore settlement and adhesion viz. the exploration phase prior to permanent attachment and that of the settled, permanently adhered spore. During the exploration stage, the swimming spores aggregate near the surface and may make physical contact with it, in some cases becoming temporarily attached via the apical papilla and spinning on the surface; a phase known as temporary adhesion.[2] The spore may then move away to explore another area of the surface or may commit to permanent attachment (settle), through the secretion of a glycoprotein adhesive that forms a pad on the surface.[3–5] The adhesive cures rapidly and the spore becomes progressively more firmly bonded to the surface.[16,17] Previous work has also shown that swimming spores are able to "detect" and respond to chemical signals.[10,11,18] The physico-chemical properties[6,7] and topography[8,9] of the surface also affect the behavior of swimming and settling spores.

There are a number of possible scenarios to consider. First, the temporarily attached spores or spores that are in the process of or have just secreted their permanent adhesive are swept across the gradient by the forces produced by the differential adhesion on the opposite sides of the apical papilla or the pads of newly secreted adhesive, respectively. There are two major difficulties with this explanation. First, for such a force to operate, as we know from the migration of liquid drops on gradient surfaces, a contact diameter greater than 100 μm is needed in order to overcome the effects due to adhesion hysteresis. Even in the absence of hysteresis, the time scale for the process is unrealistically low as the typical capillary velocity (the ratio of surface tension to viscosity) of biological cells is expected to be very low ($V^* \sim 10^{-5}$ m/s). Since a swimming spore "senses" the surface and becomes temporarily attached via its apical papilla,[2] the size of which is about 1 μm, the gliding velocity of the temporarily attached spore on the surface $[\sim V^* R(d \cos \theta / dx)]$ is expected to be in the range of a few nm/s! With such a low velocity, the spores would be unable to travel a distance of 0.5 cm while attached to the substrate over the time period (1 h) of our experimental observations. The situation hardly improves for a newly settled spore, which releases adhesive that forms a pad of 5–12 μm in diameter on hydrophobic surfaces and approximately 25 μm diameter on hydrophilic surfaces.[19] Furthermore, we know that the adhesive begins to cross-link within minutes of adhesive release.[16,17] Based on the above considerations, we may rule out the possibility of the migration of either temporarily or permanently adhered spores induced by the gradient of surface tension of the type seen with the migration of water drops on similar surfaces.

A second explanation resulting from the selective adhesion of the settled spores to the hydrophilic part of the gradient should be carefully considered. First, the observed density of spores is higher on the hydrophilic part of the gradient than on the hydrophobic part. This is in contrast to the observation on the uniformly coated control studies that shows the spores settle on the uniform hydrophobic surface at a higher concentration than on the hydrophilic surface. Nevertheless, it is also known that spores generally adhere more strongly to a hydrophilic surface than a hydrophobic surface.[20] Thus there is a possibility that the spores sense that contrast of the adhesion strengths through temporary adhesion, detach from the surface and migrate towards the region

of higher adhesion (or hydrophilic zone). Migration away from the hydrophobic zone towards the hydrophilic zone (for the case of inward gradient) would lead to a settlement density at the center of the gradient that is higher than on the hydrophilic background, but a depletion zone at the periphery of the gradient. This scenario is also consistent with the observation that there is a depletion zone at the center of the outward gradient, but a zone of accumulation at the periphery of the gradient. The above scenarios are, however, inconsistent with the previous observations with patterned SAMs of differing wettability with hydrophobic background,[6,7] in which the spores chose to settle on the hydrophobic areas of the surface. A scenario that is perhaps more consistent with both the fast response of these spores to the gradient surface as well as their nonmonotonic deposition behavior is that the swimming direction of spores is somehow biased by the underneath gradient. Different regions of the gradient surface may produce long-range differential signals that bias the directionality of their swimming. The overall process is, however, not merely that these conflicting signals discourage the spores from settling on the gradient zone; the nonmonotonic settlement behavior bears signature to a biased swimming of the spores during the exploration phase, which may discourage them from making physical contact with the surface. The overall scenario may be comparable to classical chemotaxis or chemokinesis,[11] which can moderate the locomotion of spores and bacteria in the water column, but it is not clear if this is the relevant mechanism as we are unsure of any gradient of chemoattractant in the bulk water. One cannot, however, rule out the possibility that some of the alkylsiloxane molecules of the gradient zone desorb[21] slowly in water, creating a gradient of a chemo-attractant in the bulk just above the surface. The concentration of these molecules above the glass surface should depend on the surface concentration in a nonlinear way. The nonlinearity stems from the fact that the desorption rate is, on one hand, proportional to the surface concentration, but, on the other hand, it is inversely proportional to the surface grafting density. Thus, for the outward gradient, the concentration of the desorbed silane would, at first, increase from the center towards the periphery of the gradient and then it would decrease. This picture seems consistent with the pattern of the depletion of the spores at the center of the outward gradient and an increase towards the periphery. Furthermore, the observation that the area of influence is higher than that of the original gradient may be due to the broadening of the area by diffusion. The overall picture may also be consistent with the accumulation of the spores towards the center of the inward gradient and depletion towards its periphery.

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[1]H. S. Hayden, J. Blomster, C. A. Maggs, M. J. Stanhope, and J. R. Waaland, Eur. J. Phycol. **38**, 277 (2003).
[2]M. E. Callow, J. A. Callow, J. D. Pickett-Heaps, and R. Wetherbee, J. Phycol. **33**, 938 (1997).
[3]M. S. Stanley, M. E. Callow, and J. A. Callow, Planta **210**, 61 (1999).
[4]J. A. Callow, M. S. Stanley, R. Wetherbee, and M. E. Callow, Biofouling **16**, 141 (2000).
[5]J. A. Callow, M. P. Osborne, M. E. Callow, F. Baker, and A. M. Donald, Colloids Surf., B **27**, 315 (2003).
[6]M. E. Callow, J. A. Callow, L. K. Ista, S. E. Coleman, A. C. Nolasco, and G. P. Lopez, Appl. Environ. Microbiol. **66**, 3249 (2000).
[7]L. K. Ista, L. K, M. E. Callow, J. A. Finlay, S. E. Coleman, A. C. Nolasco, R. H. Simons, J. A. Callow, and G. P. Lopez, Appl. Environ. Microbiol. **70**, 4151 (2004).
[8]M. E. Callow, A. R. Jennings, A. B. Brennan, C. E. Seegert, A. Gibson, L. Wilson, L. A. Feinberg, R. Baney, and J. A. Callow, Biofouling **18**, 237 (2002).
[9]L. Hoipkemer-Wilson, J. F. Schumacher, M. L. Carman, A. L. Gibson, A. Feinberg, M. E. Callow, J. A. Finlay, J. A. Callow, and A. B. Brennan, Biofouling **20**, 53 (2004).
[10]I. Joint, I. Tait, M. E. Callow, J. A. Callow, D. Milton, P. Williams, and M. Camara, Science **298**, 120 (2002).
[11]G. L. Wheeler, K. Tait, A. Taylor, C. Brownlee, and I. Joint. Plant Cell and Environment 1365 (2006).
[12]S. B. Carter, Nature (London) **213**, 256 (1967).
[13]M. K. Chaudhury and G. M. Whitesides, Science **256**, 1539 (1992).
[14]S. Daniel, M. K. Chaudhury, and J. C. Chen, Science **291**, 633 (2001).
[15]S. Daniel and M. K. Chaudhury, Langmuir **18**, 3404 (2002).
[16]J. A. Finlay, M. E. Callow, M. P. Schultz, G. W. Swain, and J. A. Callow, Biofouling **18**, 251 (2002).
[17]A. J. Humphrey, J. A. Finlay, M. E. Pettit, M. S. Stanley, and J. A. Callow, J. Adhes. **81**, 791 (2005).
[18]M. E. Callow, and J. A. Callow, Biofouling **15**, 49 (2000).
[19]J. A. Callow, M. E. Callow, L. K. Ista, G. Lopez, and M. K. Chaudhury, J. R. Soc., Interface **2**, 319 (2005).
[20]J. A. Finlay, M. E. Callow, L. K. Ista, G. P. Lopez, and J. A. Callow, Integr. Comp. Biol. **42**, 1116 (2002).
[21]Ongoing studies at Lehigh University provided some evidence of the desorption of surface adsorbed alkylsiloxane molecules in water. In this particular study, drainage of thin water film is investigated on a glass slide or a silicon wafer, which has circular or rectangular shaped silanized patches. As the water film drains to a thickness of about 10–15 μm, it ruptures. Examination of the optical interference fringes formed by the draining film as well as rupture dynamics and its scale dependence suggest that the alkylsiloxane molecules desorb more readily from the edges of the hydrophobic patches rather than its center.