MICROBIOLOGY

Cell-to-Cell Communication Across the Prokaryote-Eukaryote Boundary

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Bacteria are capable of complex assemblage behavior through cell-to-cell communication using diffusible chemical signal molecules that accumulate to a threshold concentration that activates target genes (*1*). This process, termed "quorum sensing," has not been shown to cross the prokaryote-eukaryote boundary. Here, we show that diffusible signal molecules produced by bacterial biofilms are exploited by the planktonic phase of a marine eukaryotic organism—the green seaweed *Enteromorpha*.

Enteromorpha produces motile zoospores that explore and settle temporarily on a surface (*2*) but will detach if it is suboptimal; i.e., suitable surfaces appear to be selected for permanent attachment. Many factors influence attachment, but we have previously shown that zoospores attach to bacterial cells in biofilms (*3*), suggesting that zoospores may sense a chemical signal produced by the bacteria.

Diverse Gram-negative bacteria produce *N*-acylhomoserine lactone (AHL) quorum sensing signal molecules (*4*). Once they reach a threshold concentration, AHLs usually activate members of the LuxR family of transcriptional regulator proteins, and the LuxR/AHL complex drives the expression of multiple target genes, including those required for AHL synthesis.

As *Enteromorpha* zoospores attach to individual bacterial cells in marine biofilms (fig. S1), we investigated whether *Enteromorpha* zoospores sense the presence of bacteria by detecting AHLs. Three approaches were taken with *Vibrio anguillarum* mutants defective in AHL production, *Escherichia coli* strains expressing AHL synthases from recombinant plasmids, and synthetic AHLs (*5*). *V. anguillarum* is a Gram-negative marine fish pathogen that forms reproducible, zoospore-attracting biofilms. This bacterium contains two linked AHL-dependent quorum sensing circuits (VanIR and VanMN), and mutants defective in AHL production have been constructed (*6*). Wild-type *V. anguillarum* biofilms strongly enhanced zoospore settlement compared with controls (Fig. 1A). However, no density-dependent stimulation of attachment was observed with a *vanM* mutant that does not produce *N*-hexanoylhomoserine lactone (C6-HSL) and *N*-(3-hydroxyhexanoyl) homoserine lactone (3-OH,C6-HSL) and is also deficient for *N*-(3-oxodecanoyl) homoserine lactone (3O,C10-HSL) (*7*). Zoospore attachment was stimulated by another mutant (*vanI*), which produces C6-HSL and 3OH,C6-HSL (*7*) but not 3O,C10-HSL. The involvement of all three AHLs was confirmed with the *vanIM* double mutant, which is AHL-negative and failed to stimulate zoospore attachment (Fig. 1A). These results indicate that *Enteromorpha* zoospores sensed and responded to AHLs produced by *V. anguillarum*.

To exclude the possibility that unidentified features of the mutants affected zoospore settlement, we carried out assays using *E. coli* strains lacking AHL synthases unless transformed with *vanI*- or *vanM*-expressing plasmids (*6*). Zoospore settlement was only enhanced on biofilms of strains expressing recombinant *vanI* or *vanM* (Fig. 1B) and showed that spores sensed 3O,C10-HSL, as well as 3OH,C6-HSL and C6-HSL.

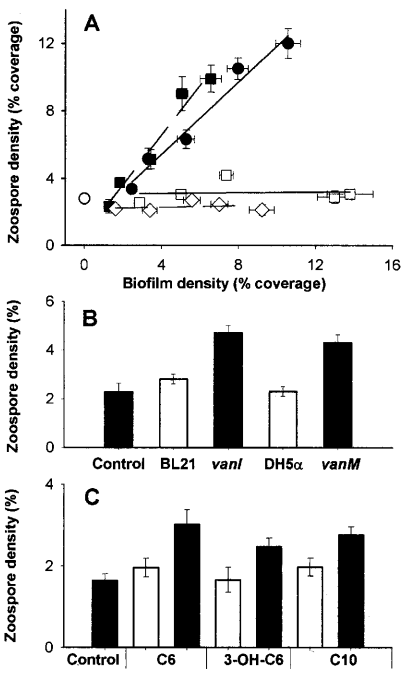

Fig. 1. (**A**) Settlement of *Enteromorpha* zoospores on wild-type *V. anguillarum* and mutants, expressed as percentage of surface covered. ○, control surface (clean cover glass); ●, wild type; □, *vanM* mutant; ■, *vanI* mutant; ◇, *vanIM* mutant. (**B**) Attachment of zoospores to *E. coli* strains BL21 and DH5α with and without the insertion of plasmids expressing *vanI*—producing 3O,C10-HSL—and *vanM*—producing both C6-HSL and 3OH,C6-HSL, respectively. There was no enhancement of attachment to either strain containing the vector plasmids alone. (**C**) Settlement of zoospores is not enhanced in the presence of three AHLs that have been treated to open the lactone ring structure (white bars) but is restored when the ring is closed (black bars). All error bars indicate ±2 SE.

Finally, the direct effect of AHLs on zoospore settlement was tested with synthetic AHLs (*6*). AHLs with acyl-chain lengths from C6 to C14 increased zooplankton settlement; only *N*-butanoylhomoserine lactone (C4-HSL) failed to enhance settlement. Moreover, the response was specific to functional AHLs. Alkaline pHs destroy AHL signaling properties by opening the lactone ring; ring-opened AHLs failed to enhance zoospore settlement (Fig. 1C). Chemotaxis requires the ability to detect concentration gradients; the addition of three different AHLs to the seawater covering biofilms of wild-type *V. anguillarum* removed the gradient and abolished enhanced zoospore settlement.

Bacterial biofilms play an important role in the development of algal communities. We have shown that zoospores of a eukaryote alga can exploit a bacterial sensory system. This ability may influence the selection of attachment sites of *Enteromorpha* and may be important to its ecology. The way in which zoospores sense and respond to AHLs is unknown. In the case of *Enteromorpha* settlement, interrupting AHL signaling may provide a means of controlling this important fouling organism.

References and Notes

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Supporting Online Material
www.sciencemag.org/cgi/content/full/298/5596/1207/DC1
Methods
Fig. S1

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