Cell-to-cell communication across the procaryote / eucaryote boundary

Ian Joint,1† Karen Tait,1 Maureen E. Callow,2 James A. Callow,2
Debra Milton,3 Paul Williams,4,5 Miguel Câmara4

1Plymouth Marine Laboratory, Prospect Place, Plymouth, PL1 3DH, UK. 2School of Biosciences, University of Birmingham, Birmingham, B15 2TT, UK. 3Department of Molecular Biology, Umeå University, S-901 87 Umeå, Sweden. 4School of Pharmaceutical Sciences, University of Nottingham, Nottingham, NG7 2RD, UK and 5Institute of Infections and Immunity, University of Nottingham, Queen’s Medical Centre, Nottingham NG7 2UH, UK.
† To whom correspondence should be addressed. E-mail I.Joint@pml.ac.uk

Supporting Online Material

Methods

Experiments with Vibrio anguillarum

Cultures of V. anguillarum WT and the V. anguillarum mutants vanI, vanM and vanIM were grown to stationary phase in Tryptic Soy Broth (Difco). Cells were harvested by centrifugation (10,000 g for 10 min), washed and resuspended in sterile sea water. Different cell densities of biofilm were obtained by adding varying quantities of cell suspension to filter-sterilized sea water and immersing cover glasses supported in stainless steel stands (S1). Biofilm cultures were incubated for 48 h and washed in sterile sea water to remove any non-adhered bacteria before zoospore settlement assays. Enteromorpha zoospore attachment was assessed using the method of Callow et al. (S2). Suspensions of freshly released zoospores were added to Petri dishes containing experimental biofilms and clean
cover glass controls. After incubation for 1 h in the dark, the cover glasses were rinsed in sea water to remove non-adhered zoospores, fixed with glutaraldehyde and stained with dilute carbol fuschin. The proportion of the surface area covered by bacteria and by zoospores was determined with microscope image analysis (S3), using a Zeiss Kontron KS 300 Imaging system.

**Experiments with *E coli* strains.**

Biofilms of the *E. coli* strains were prepared using similar methods to those used for *V. anguillarum*. The initial bacterial inoculum was grown in Luria broth (Bacto Tryptone, 10 g l\(^{-1}\); Bacto Yeast Extract, 5 g l\(^{-1}\)), and the salinity of the sea water in the biofilm culture vessel and zoospore settlement assay was reduced to 25. At this salinity, zoospores are very active and *E. coli* biofilms developed well but at higher salinities, *E. coli* growth was poor.

**Experiments with synthetic AHLs**

Three AHLs, 3-oxo-C10-HSL, 3-hydroxy-C6-HSL and C6-HSL, were synthesized, purified and characterized as described previously (S4, S5). A 1\% agarose / distilled water solution was used as a support matrix for the AHLs, each dissolved at 50µM. A consistent thin coating of agarose / AHL was applied to cover glasses using a mould. The coated cover glasses were immersed in zoospore suspension and zoospore attachment was measured by direct count using a microscope.

The signaling properties of AHLs are inactivated at high pH. This pH dependent effect is due to lactonolysis. \(^{13}\)C nuclear magnetic resonance spectroscopy has confirmed opening of the homoserine lactone ring (S6). The effect is reversed by acidification to pH 2; however, once opened by increased pH, the ring does not close when the pH is brought back to that of sea water.
Fig. S1 Attachment of zoospores of Enteromorpha to biofilms of Vibrio anguillarum wild type (WT) and a vanM mutant (Van M) which produces neither N-hexanoyl-L-homoserine lactone (C6-HSL) nor N-(3-hydroxyhexanoyl)-L-homoserine lactone (3-hydroxy-C6-HSL). As the AHLs produced on vanM are also required for the production of N-(3-oxodecanoyl)-L-homoserine lactone (3-oxo-C10-HSL), the vanM mutant is deficient for all three AHLs.

References


