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13. ABSTRACT *(Maximum 200 words)*

This work indicates that the distribution and abundance of a class of marine micro-organisms, the symbiotic luminous bacteria, are controlled by the dynamics of their relationship with their host. Such a conclusion constitutes the first time that the ecology of any marine bacterium could be related to a predictive biological factor. This study also points to the presence of a significant population of typically culturable V. fischeri cells in seawater that have entered a non-culturable state from which they can be recovered only through an association with a specific animal tissue. Such a phenomenon changes the way we view the initiation of the light organ symbiosis. However, of even broader interest, it suggests that at least a portion of the 99.9% of bacteria in seawater that can not be cultured may not be unknown or new species, but in fact are well-known, typically culturable bacteria that are awaiting a specific environmental cue that signals these cells to re-enter a proliferative stage in their cell cycle.

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FINAL TECHNICAL REPORT

The function of this award was to support the graduate student responsible for the work proposed in project # N0014-91-J-1670. Specifically, he has accomplished the results summarized below in three aspects of our studies.

Distribution and abundance of non-visibly luminous *V. fischeri*

As a result of earlier work from our lab we reported that symbiotic bacteria inhabiting the light-emitting organ of *E. scolopes* are distinct from typical *V. fischeri* organisms in that they are not visibly luminous when grown in laboratory culture. Therefore, the abundance of these bacteria in seawater samples cannot be estimated simply by identifying them among luminous colonies that arise on nutrient agar plates. Instead we have used *luxR* and PCR-generated *luxA* gene probes (developed by C. Wimpee and K. Nealson) to identify both luminous and non-visibly luminous *V. fischeri* colonies by DNA-DNA hybridization. We demonstrated that the probes were specific, hybridizing at least 50 to 100 times more strongly to immobilized DNAs from a diversity of isolates of *V. fischeri* than to DNA from pure cultures of other related species. Thus, even non-visibly luminous *V. fischeri* colonies could be easily identified and isolated among colonies obtained from natural seawater samples by their probe-positive reaction. This approach was used to demonstrate that there were no significant differences in the abundance of visibly luminous ("F-type") *V. fischeri* colony-forming units (CFUs) in water samples obtained either within or distant from populations of symbiotic squid (1 to 3 CFU/ml). However, non-visibly luminous ("S-type") *V. fischeri* were found in abundance (up to 900 CFU/ml) only in seawater collected from within the squid's habitats. Further, these planktonic strains were shown to be fully competent in initiating a light organ symbiosis with axenic juvenile squids.

Population genetics of symbiotic *V. fischeri*

Initial studies have been completed to determine the efficacy of various molecular measures of genetic diversity between strains *V. fischeri* isolated both from planktonic niches, and from the light organs of geographically and temporally distant populations of squids. Analyses of 15 different chromosomal DNA restriction fragment length polymorphisms (RFLPs) and 8 multilocus enzyme electrophoresis patterns revealed the presence of two distinct groups of planktonic Hawaiian *V. fischeri* isolates (S-type, and F-type), only one of which (S-type) was indistinguishable from Hawaiian squid symbionts. All of these Hawaiian strains resembled each other more than they resembled *V. fischeri* isolated from the light organs of the Japanese squid *Euprymna morsei*. These results suggest that, as is the case within the various well-studied species of symbiotic N_2-fixing rhizobia, there may be biovars of symbiotic *V. fischeri*. The level of resolution inherent in these studies did not indicate any population structure within the strains of Hawaiian symbiotic *V. fischeri*. Preliminary data obtained both by RFLP analyses and by sequencing PCR-generated products of *V. fischeri gapA* genes suggest that this approach is not only easier, but has considerably more resolution than our previous efforts, and may provide genetic information at the population level.

Comparisons of extrachromosomal DNA from Hawaiian isolates revealed the presence of an extensive degree of similarity between plasmids carried by both F-type and S-type strains. Within Hawaiian waters these plasmids appeared to be confined exclusively to *V. fischeri*: colony hybridization screenings of several thousand CFUs from natural Hawaiian seawater collected over several years gave no positive hybridization to these plasmid sequences (except to those colonies that were identified as *V. fischeri*). In addition, the plasmids appeared to be confined to Hawaiian *V. fischeri*, and were absent among all CFUs arising from seawater collected in either Woods Hole, MA or Southern California, even though several hundred *V. fischeri* CFUs were present in these samples.

Competition studies between symbiotically-competent strains of *V. fischeri*

Both F-type and S-type *V. fischeri* can be isolated from seawater in the squid host's habitats, and both types will colonize the newly hatched, axenic juvenile squid in laboratory infection assays; however, only S-type have ever been isolated as light organ symbionts. One factor at least partially responsible for this situation is that the S-type bacteria are as much as 1000-times as abundant in the seawater surrounding the newly hatched squid, and so are stochastically more likely to initiate the symbiotic association. This quantitative dominance is due to the active expulsion of S-type bacteria by host animals at rates of between 10^5 to 10^7 cells per day (depending on host size). However, other factors are apparently operating to prevent F-type bacteria from establishing themselves in a symbiosis. Mixed colonization infection assay experiments have suggested that S-type bacteria consistantly outcompete F-type strains during the initiation and maintenance of the symbiotic infection. These studies demonstrate the close interdependence between the life cycle of the host animal and the ecological cycle of this species of luminous marine bacteria. In addition, they indicate the suitability of the squid/luminous bacterium symbiosis as a model system to examine the genetic and physiological bases underlying the phenomena of intra-specific competition and exclusion during symbiotic colonization.

Publications and Abstracts

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5. Lee, K.-H., and E.G. Ruby. Effect of the squid host on the abundance and distribution of symbiotic *Vibrio fischeri* in nature (submitted to: **Appl. Environ. Microbiol.**).