Contribution by Symbiotically Luminous Fishes to the Occurrence and Bioluminescence of Luminous Bacteria in Seawater

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Abstract. Seawater samples from a variety of locations contained viable luminous bacteria, but luminescence was not detectable although the system used to measure light was sensitive enough to measure light from a single, fully induced luminous bacterial cell. When the symbiotically luminous fish Cleidopus gloriamaris was placed in a sterile aquarium, plate counts of water samples showed an increase in luminous colony-forming units. Luminescence also increased, decreasing when the fish was removed. Light measurements of water samples from a sterile aquarium containing Photoblepharon palpebratus, another symbiotically luminous fish, whose bacterial symbionts have not been cultured, showed a similar pattern of increasing light which rapidly decreased upon removal of the fish. These experiments suggest that symbiotically luminous fishes release brightly luminous bacteria from light organs into their environment and may be a source of planktonic luminous bacteria. Although planktonic luminous bacteria are generally not bright when found in seawater, water samples from environments with populations of symbiotically luminous fish may show detectable levels of light.

Introduction

Planktonic luminous bacteria are ubiquitous in seawater. They can usually be isolated on agar media in numbers ranging from $10^2$ to $10^4$ per liter [7-9, 12-14, 16]. In a given environment, major fluctuations in species composition may be seen on a seasonal scale [9, 10, 12, 13, 16]. The factors responsible for these variations are not yet completely understood, but they have been postulated to be due to the physical and chemical properties of the environments studied.

Luminous bacteria emit light in laboratory culture and in symbiotic associations. However, the question of whether or not planktonic luminous bacteria emit light in situ has rarely been examined [1]. In this paper we present evidence that although planktonic luminous bacteria were ubiquitous in the environments we examined, they were not luminescent.
The same species of bacteria that are found as planktonic forms can also be isolated from one or more of a variety of symbiotic associations (saprophytic, gut symbiotic, light organ symbiotic, or parasitic) [3, 7, 8, 10, 11]. In these associations they can attain very high numbers (10⁹ per ml or more) and be visibly luminous.

We report here the relationship between symbiotic and planktonic niches in 3 symbiotically luminous fishes: Photoblepharon palpebratus (family Anomalopidae), Cleidopus gloriamaris and Monocentris japonicus (both in the family Monocentridae)[2]. These fishes occupy shallow water marine habitats; the anomalopid is a tropical species, whereas the monocentrids are subtropical and temperate species [5]. The specific symbiont of the 2 monocentrids is Vibrio fischeri [11]; that of P. palpebratus has not been cultured or identified. Structural studies of the suborbital light organs of P. palpebratus and the light organs located at the anterior end of the lower jaw of M. japonicus show that the light organs communicate with the surrounding seawater via pores on the surface [4, 15].

The relationship between these associated populations and the planktonic forms is not known, although it has been suggested both that gut symbionts may contribute significantly to populations of luminous bacteria in seawater [10] and that light organs of certain marine fishes may serve as a specific source of some luminous bacterial species [3, 14]. Fish gut tracts contain luminous bacteria but cell densities and relative species abundance may be quite variable. In some species of fishes, species of luminous gut symbionts have been shown to vary seasonally [10]. On the other hand, light organs contain a fixed population of a pure culture of luminous bacteria which may provide a continuous input of a given species to the environment. We have focused our research on release of bacteria from light organs, and our results suggest that this indeed occurs. In addition, our results suggest that released symbionts have the potential to produce measurable levels of light in habitats heavily populated by symbiotically luminous fishes.

Materials and Methods

Collection of Samples, and Measurements of Light and Growth of Bacteria

Seawater samples were collected in a variety of ways (Niskin bottles, sterile syringes, open bottles, continuous pumping, and sterile tubes), from a number of sites and depths (Table 1), and throughout the day and night. The water was passed through a 10 micron mesh net to exclude dinoflagellates and placed in sterile glass scintillation vials that had been previously kept in the dark to minimize fluorescence. These samples, ranging from 1-20 ml, were then viewed and counted by an E.M.I. 9635 photomultiplier tube in the chamber of a modified JRB-ATP photometer (S.A.I. Industries, Sorrento Valley, CA). In cases in which luminescence was seen, a second sample was obtained and counted and the pH then adjusted to approximately 4 by the addition of 1 M HCl, and recounted. This allowed distinction between bacterial light emission, which is characteristically sensitive to pH of less than 6 [2], and delayed fluorescence or phosphorescence of the seawater, which was common in the near-shore samples taken during the day.

All samples except those treated with HCl were then used for determination of bacterial populations. Two methods of enumeration and isolation of luminous bacteria were used, depending on the volume of the sample to be examined: either 0.1 ml of seawater was spread directly onto