論文内容の要旨

論文題目 DIVERSITY OF LIGHT EMISSION SPECTRA OF MARINE LUMINOUS BACTERIA

（海洋性発光細菌の発光色多様性）

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Luminous bacteria are a group of microbes emitting bluish-green light as a result of luciferase reaction. Except for a few group, they are unique to marine environments and ubiquitously present in the ocean including deep sea. They have drawn attentions of microbiologists since the first description in 19th century. Why do most of them live in the sea? For what do they emit light? When and from which microbes, is the bioluminescence originated? These basic questions are still left unanswered.

Luminous bacteria are isolated from water column, particulate matter and the light organ of some fish. They are also easily found in the gut of marine animals. Such association with animals suggests that the interaction with animals may at least partly explain why they emit light. This further raises a question whether animals recognize only the presence of light or they recognize its color. There is, however, no microbial ecological work related to this intriguing question. In this work, I made two basic hypotheses; first, there are variations in the bacterial light emission spectra, and second, such spectra have ecological implications. The first purpose of this work was to verify these hypotheses. My second purpose was to clarify how bioluminescent spectra were related to the phylogeny of luminous bacteria. Finally, I intended to overview the origin and gene transfer among

marine luminous bacteria. Phylogenetic, physiological, and biochemical analyses were done for the 777 luminous bacteria.

Chapter 1.

The distribution pattern of luminous bacteria of different phylogenetic group and their light emission spectra were investigated. Altogether 777 isolates were obtained from coastal and open ocean from surface to deep sea environments, and identified by using 16S rRNA sequences analysis. The data showed that *Vibrio* species are dominant at seashore and surface water of coastal area. *Photobacterium* species were mainly found in both coastal area and open ocean. *Aliivibrio* species were isolated from seashore in only winter season. These distribution patterns of each genus seem to be mainly controlled by sea temperature. The light emission spectra consist of 5 distinguishable types with different maximum wavelength and each type was unique to certain phylogenetic group (*Vibrio*, λ_{max}≈473 and 482 nm; *Photobacterium*, λ_{max}≈479 and 488 nm; *Aliivibrio*, λ_{max}≈485 nm). Among them, three types (λ_{max}≈482, 485 and 488 nm) showed Gaussian spectra, whereas the other two (λ_{max}≈473 and 479 nm) showed asymmetric one and have shorter maximum wavelength. In addition, the FWHM (full width at half maximum) of the former was more than 80 nm whereas those of the latter two were less than 75 nm. Considering on the related literature information, I concluded that the latter two were the blue-shifted light emission type. The distribution of the strains with blue-shifted light emission type did not seem to depend on the seawater temperature but rather on the light condition of the environment. These results verify my first hypothesis that there are variations of light emission spectra. Furthermore, It was revealed that those differences were reflected to the phylogenetic positions of each type.

Chapter 2.

At present, 19 species among 4 genera (*Vibrio, Photobacterium, Aliivibrio,* and *Shewanella*) have been known as marine luminous bacteria. During the course of investigation described in Chapter 1, the inconsistency and confusion of systematics of luminous bacteria became clear. Without robust and reliable identification scheme, it is difficult to deduce the phylogenetic position of my isolates and further discuss on the evolutionary processes. In this chapter, I tried to apply the latest molecular phylogenetic approach and identify my 5 isolates that seem to be ecologically or phylogenetically important. As a result, 5 new species, i.e., *V. azureus, V. sagamiensis, P. aquimaris, A. marinus,* and *A. lajollensis* were newly described and proposed. It is noteworthy that about 60% isolates at Sagami

Bay belonging to new species.

Chapter 3.

The variations in the luminescent spectra may be ascribed to the structural differences of the molecules involved in the light emission. As they are encoded by *lux* genes, it is assumed that the variations may be correlated with the phylogeny of these genes. Therefore, luciferase alpha subunit gene (*luxA*) sequence and light emission spectra were analyzed concomitantly. Consequently, it was revealed that each species, except for *P. leiognathi*, formed individual clade and the strains sharing the same clade have same light emission spectra. Furthermore, the presence of the genes encoding the accessory fluorescent protein of *P. phosphoreum* was confirmed for all strains with blue-shifted light emission in the genus *Photobacterium* by PCR. Therefore, I concluded that light emission spectra were primarily determined by the lineage of phylogeny of *luxA*. The blue-shifted light emission among strains in the genus *Photobacterium* is explained by the presence of an accessory fluorescent protein. For other genera, however, it remains to be elucidated.

Chapter 4.

Chapter 1 and 3 clarified the variation of light emission spectra among marine luminous bacteria. So far, this is the first observation of blue-shifted light emission within genus *Vibrio*. Although the blue-shifted light emissions in *Photobacterium* seem to be explained by an accessory fluorescent protein, there has been no report on the presence of similar functional protein in the genus *Vibrio*. Therefore, I tried to detect an accessory fluorescent protein as substance responsible for blue-shifted light emission in *Vibrio azureus*, which is newly proposed species in Chapter 2. As a result, a blue fluorescent protein, which had a fluorescence spectrum similar to that of the *in vivo* light emission spectrum of the strain, was purified by the biochemical procedures using liquid chromatography analyses. Consequently, the data suggested that all blue-shifted light emissions of luminous bacteria are due to accessory fluorescent proteins.

Chapter 5.

The previous chapters clarified the correlation between luminescent spectra and their phylogenetic positions. On the other hand, luminous bacteria generally do not make single clade, but rather scattered in the phylogenetic tree. Taken together, it seems reasonable to assume that *lux* genes were acquired or lost during the process of evolution in *Vibrionaceae*. Also, it is expected that if any gene transfer events are involved, there may be a certain point that the evolution of species and that of *lux* genes do not agree with each other. In order to clarify the evolutionary process of bioluminescence among marine bacteria, I compared the differences between the phylogenetic trees constructed by both *luxA* and multilocus house-keeping genes. If any strain acquired luciferase gene from another strain by horizontal transfer, incongruence between the phylogenies of *luxA* and housekeeping genes may be seen among these strains. Results indicated that the scattered presence of *lux* genes in the phylogenetic tree may be explained by the gene deletion during the evolutionary process. However, there were also some cases which are explained reasonably if a gene transfer event is assumed. In addition, I examined the presence and phylogenetic analyses of genes encoding the accessory fluorescent protein. It is indicated that the accessory fluorescent protein of *V. azureus* was acquired from genus *Photobacterium* by horizontal transfer. These results imply that the diversification of luminous bacteria and their emission spectra are well explained by the evolutionary steps of both species and the related genes. What kind of actual event caused the gene loss and/or gene transfer remain to be elucidated.

This doctoral thesis is the first intensive examination of light emission spectra of marine luminous bacteria. I had made two hypotheses, i.e., first, there are variations in the bacterial light emission spectra, and second, such spectra have ecological implications. As was described in Chapter 1, there are 5 distinct types of the spectra, so the first one was confirmed. The second hypothesis was partly supported by the two findings. First, the strains with blue-shifted spectra showed characteristic distribution in the sea. Second, the luminous bacteria with blue-shifted spectra are quite widely distributed in marine environments and this shift seems to be attained by the accessory fluorescence protein. It is difficult to assume that those bacteria synthesize such compounds without any advantage. Then what are the ecological advantages of the modulation? There are two possibilities that are not exclusive each other. First, the bacteria of which luminescence penetrate most in the sea have selective advantages. Second, fish recognize the slight difference of the luminescence and preferentially ingest some types. My work at least supports the former possibility, and the latter needs fish physiologist to confirm it. In any case, these possibilities have never pointed out before because there has been no work on the luminescent spectra before. This work shows the ecological implication of not only luminescence spectra but also bacterial bioluminescence itself.