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学 位 論 文 題 目

Distribution and characteristics of algicidal and growth-inhibiting bacteria in coastal sea and
seagrass beds

(沿岸海域及びアマモ場における殺藻細菌と増殖阻害細菌の分布と特性)

Harmful algal blooms (HABs) can lead to the detrimental effects on co-occurring organisms through oxygen depletion or toxin productions and alter food web dynamics that could eventually induce illness or death on marine lives such as dolphins, manatees, sea lions and even human. Recent researches are indicating the increases in the frequency, severity and geographic distribution of HABs in the world due to the eutrophication and climate change, etc. Therefore, developments of effective countermeasures against the HAB events are crucial. However, no practical measures of HABs control are established except clay dispersion in a limited extent in spite of global expansions of HABs. The control of HABs requires direct interventions with ecosystems which easily lead to public concerns and oppositions. Therefore, a measure that is as environmentally friendly as possible is desired for the future establishment of HABs control in the world. Biological controls are thought to be the least impact to ecosystems in the present circumstances in that it harnesses natural living organisms. In this dissertation, new prospective of phylogenetic and ecological characteristics of natural living bacteria that could potentially be utilized to suppress HABs in coastal sea, seagrass and macro-algae beds were investigated and discussed through chapter II to chapter V.

Dynamics of algicidal bacteria (AB) and growth-inhibiting bacteria (GIB) in the coastal area of Yatsushiro Sea, Japan were revealed in Chapter II. These bacteria showed a close relationship with chlorophyll *a* fluctuation during a period when no HABs were present indicating new ecological roles of those growth-limiting bacteria (AB and GIB) in regulating phytoplankton populations in natural environments during a period when no HABs were present, possibly acting as homeostasis of coastal sea to a certain extent. Moreover, much less frequencies and densities of AB were detected in offshore waters than the coastal water implied the origin of these bacteria are primarily in nearshore areas.

In Chapter III, the temporal variations of algicidal and growth-inhibiting bacteria were investigated at the seagrass bed in Miyazu Bay, Kumamoto Prefecture, Japan. It was clarified that

AB were most abundant in summer with the density reaching more than ten million CFU per 1 g of seagrass blade which was thousand times higher than the density in the seawater. Growth-limiting bacteria (AB and/or GIB) were constantly present in the seawater at the seagrass bed through sampling period except the last sampling day (the end of September) when most of seagrasses were lost by the withering. Changes in phytoplankton density and compositions at the seagrass bed shifted low densities with pennate diatoms dominated to high density with centric diatom domination corresponding with seagrass loss at the site. It was suggested that the loss of habitat for these bacteria influenced phytoplankton community structures in the seawater.

In Chapter IV, the distributions of AB and GIB associated with two seagrass species *Z. marina*, *Z. japonica* and several macro-algal species were examined throughout five different basins in Puget Sound, WA, USA. Between hundred to ten thousand times higher densities of growth-limiting bacteria were observed in the biofilm of two seagrass species *Z. marina* and *Z. japonica*, and one green alga *U. lactuca* in Puget Sound compared to the densities studied for nearshore water of Yatsushiro Sea, Japan (Chapter II). The distribution and densities of these bacteria associated with seagrass and macroalgae were revealed and described first time in USA. In particular, this is the first report showing the attachment of GIB on the biofilm of *Z. japonica*. These states of seagrass beds as home of AB and GIB are probable to be common phenomena globally.

In Chapter V, how predators and competitors of AB and GIB influence their dynamics in the natural seawater at the seagrass bed were investigated to assess capability of these bacterial assemblages for controlling the bloom of fish-killing raphidophyte *C. antiqua*. Bacterial communities living in the seawater at the seagrass bed and biofilm formed on the seagrass blade effectively suppressed *Chattonella* bloom under the presence of their competitors and predatory organisms. Phylogenetic analysis suggested that considerable numbers of *Chattonella* growth-limiting bacteria isolated from the seagrass biofilm and seawater at the seagrass beds are the decomposers of lignocellulosic compounds that are likely to be abundant at seagrass beds. The direct comparison of 16S rRNA sequences of growth-limiting bacteria isolated from *Z. marina* biofilm and seawater at seagrass bed (St. 1) and nearshore water (St. 5) revealed that 4 different strains of growth-limiting bacteria had identical gene sequences to the isolates from seawater at seagrass bed (St. 1) and also at nearshore water at St. 5, respectively. Notably, growth-limiting bacteria (strain ZM101) belonging to the genus of *Phaeobacter* (*alphaproteobacteria*) isolated from the biofilm of *Z. marina* showed 100% similarity of 16S rRNA gene sequence with 4 strains and 3 strains of growth-limiting bacteria isolated from seawater at Sts. 1 and 5, respectively. These results give a quite reasonable answer to their origins as seagrass biofilms and/or seawater at seagrass beds.

Seagrass beds are the one of the most important coastal constituents, not only serving as critical habitat for marine and estuarine animals, providing nursery, migratory grounds and food resources. In this dissertation, it was indicated that seagrass beds provide a new important role as the nursery of these algal growth-limiting bacteria. These new findings give more values to the vital nearshore habitat that could also potentially enhance the movements toward the protection and restoration of seagrass beds which may be applied to be environment-friendly HABs control.