CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

Gram stain has been using for a century as an essential basic technique in the bacterial microbiology. As being evident from the examined results of Gram stain of all identified bacteria being cited in the most authorized manual on taxononomical bacteriology "Bergey's Manual of Determinative Bacteriology", Gram stain is still one of the most important methods in microbiology.

However, it has been believed to be difficult to use the Gram stain in the field studies for ecological analysis, partly because the results could be expected were only dichotomous (Gram negative or Gram positive), and partly because that the determination of stainability was depended on the eyesight judgment.

Because of the recent progress of photometrical technology, on the other hand, the determination of stainability has become possible to express numerically. This kind of progress on the evaluation system for Gram stain can be expected to bring about a revolutionary device in the ecological and environmental microbiology. It made possible to express the stainability in much detail numerically, in the range of −1 to +1 with the third decimal. By using this new system, some scientific facts have been raised, so that the possibility of GSI usage has been expanded to ecological analyses of natural bacterial communities. While the progress of GSI determination of microorganisms by the photometrical method, it has been found clearly that the

stainability of each cell, even isolated strains such as *E. coli* and *B. subtilis* shows some variation. Also, its different stainability of bacterial strains has been shown to take place principally at different growth phase. However, the characterization of an isolated strain or a natural bacterial community can be possible by the determination of GSI histogram of a strain or a community with 300 bacterial cells. Consequently with the characterization as fingerprint, the GSI histogram can be useful not only for the taxonomical featuring of a natural community but also for the holistic index of an environmental situation.

The *in situ* experiment using our new "gradostat" has supplied reliable evidence to evaluate different natural bacterial communities in aquatic environments by the practical use of GSI. A nutrient gradient formed in this semi-enclosed equipment could be useful to evaluate experimentally the correlation between GSI of the natural bacterial community and the organic nutrient in the natural environment. The results in Chapter 2-2 showed not only the usefulness of GSI histogram for natural bacterial community but also the nutrient effect onto bacterial community at the natural condition.

Analyzing the GSI stainability of natural bacteria at the natural environment without artificial culture or any selective procedure in the laboratory can be worthy of being emphasized the importance of such *in situ* experiment in the ecological study.

The author has demonstrated the possibility of Gram stain index (GSI) for structural analyses of natural bacterial communities in various aquatic environments. These studies show the applicability of this *in situ* experimental system for the monitoring of species succession in the bacterial community by the nutrient perturbation.

The Gram stain index (GSI) is originally proposed for characterizing a stainability of Gram stain of both isolated strains and natural microbial community to be used an holistic indicator of aquatic environments and a method to analyze microbial structure. It can be applied also evaluating a change of water quality in the aquatic environment. As the author presented the evidence in Chapter 2-2 ii, population successions of aquatic bacteria occur within such a short period as a day, so that the species succession in a natural bacterial community can be a good indicator of pollution impact upon an aquatic environments. Other possible applications of GSI for monitoring the aquatic environment are listed as follows:

1) Eutrophication because of

    i) Municipal waste

    ii) Waste from livestock industries and products

    iii) Aquaculture

    iv) Harmful side effect of wastewater treatment

2) Other pollutions because of

i) Oil leakage from tankers in the seas and oceans

ii) Chemicals

Halophilic Archaea showed typical GSI profiles on isolated strains and natural community. The GSI profile of a standard strain of halophilic Archaea, *H. salinarum*, showed distinctly different profiles from those of Bacteria, However, it is much more similar to those of other isolates of halophilic Archaea and even the microbial community in a hypersaline lake. Furthermore, clear relationship between GSI and dominant halophilic Archaea was observed in the field investigations in Egypt and China. These results must imply that GSI would be able to clarify if a community is dominated by halophilic Archaea.

As well as the field investigation, the laboratory experiments showed interesting results as another typical extreme halophilic Archaea, *Natronomonas pharaonis* (JCM 8858), showed almost the same GSI profiles at every different concentration of Na^+ and Mg^+ in the medium until the critical concentration for their survive (Fig. 5-1). In addition to former result, *N. pharaonis* and *H. salinarum* showed very similar GSI profiles irrespective of their growth stage in optimal media for them (Fig. 5-2 and 5-3). Therefore, it can be concluded that GSI makes easier to characterize the microbial

community structure in very extreme saline water.

For evaluation of the GSI of the microbial community as an environmental index for worldwide use, it is important to make seasonal investigations at various aquatic environments with different types of trophic level, in both freshwater and sea water sediment is also an important sample source, because pollutants tend to accumulate there. Extreme environments such as hot spring, deep sea as well as hypersaline lakes must also to be considered as important investigation sites because of following reasons:

1) Increased awareness of the widespread distribution of extreme environments.
2) Increased recognition of the commercial value of organisms from extreme environments and their physiological products.
3) General recognition that Earth's earliest organisms may have been originated in extreme environments.

The GSI method is still under consideration to be improved more, especially because of applying for the field investigations. Developing of portable staining equipment will be required to get real-time and stable results. To get more useful biological informations, it is desirable to combine with other methods such as FISH and some staining methods like FDA, CFDA, and EB. By the combination of these

methods with GSI, the staining procedures will supply much more useful informations for the analysis of microbial activity in addition to that of the species structure of microbial community in natural aquatic environment.

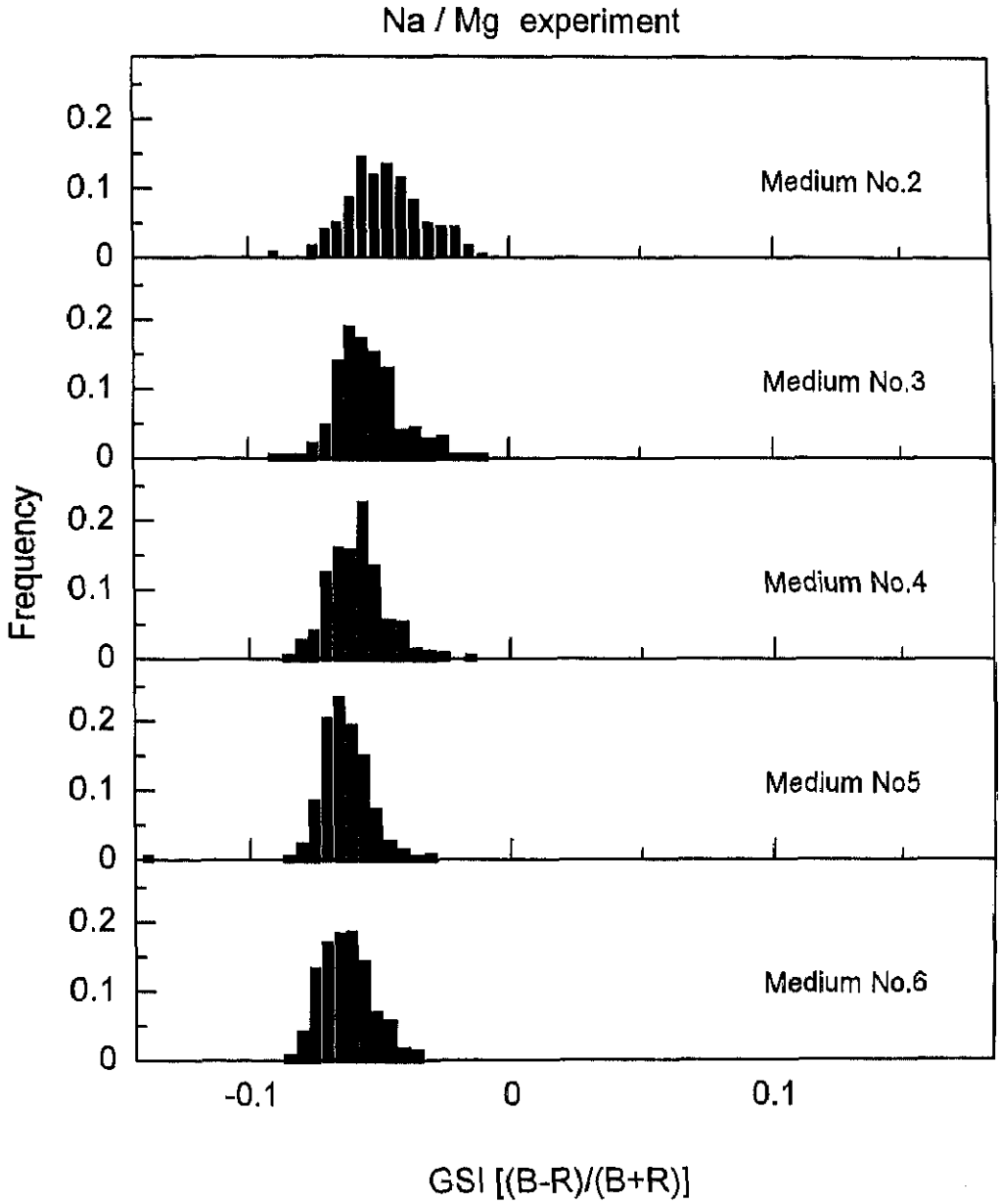

Fig. 5-1. GSI profiles of *N. pharaonis* cultured in different ratio of NaCl to $MgSO_4$ as follows; Medium 2 = 1.35, Medium 3 = 2.22, Medium 4 = 4.12, Medium 5 = 11.41, Medium 6 = 100.

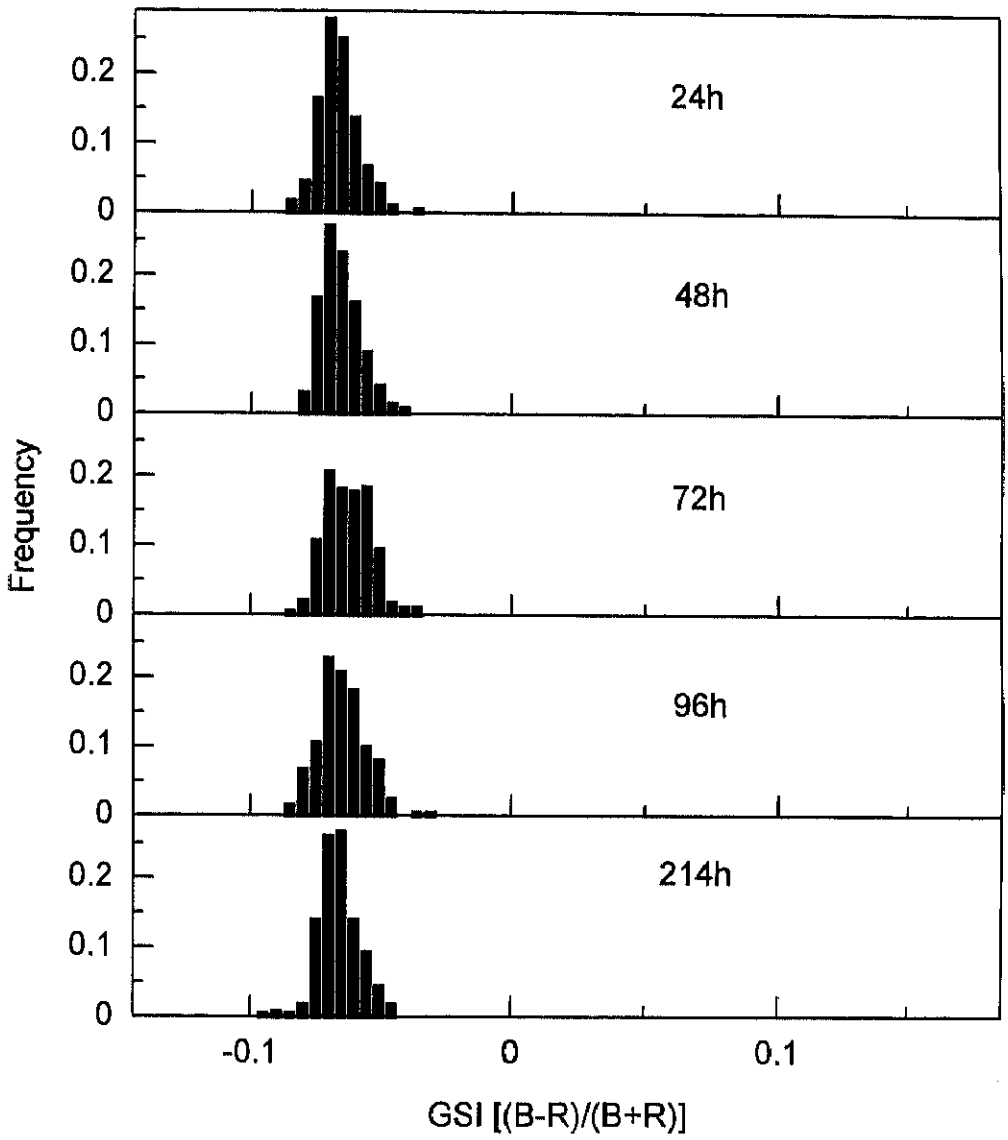

Fig. 5-2. GSI profiles of *N. pharaonis* at different growth phases

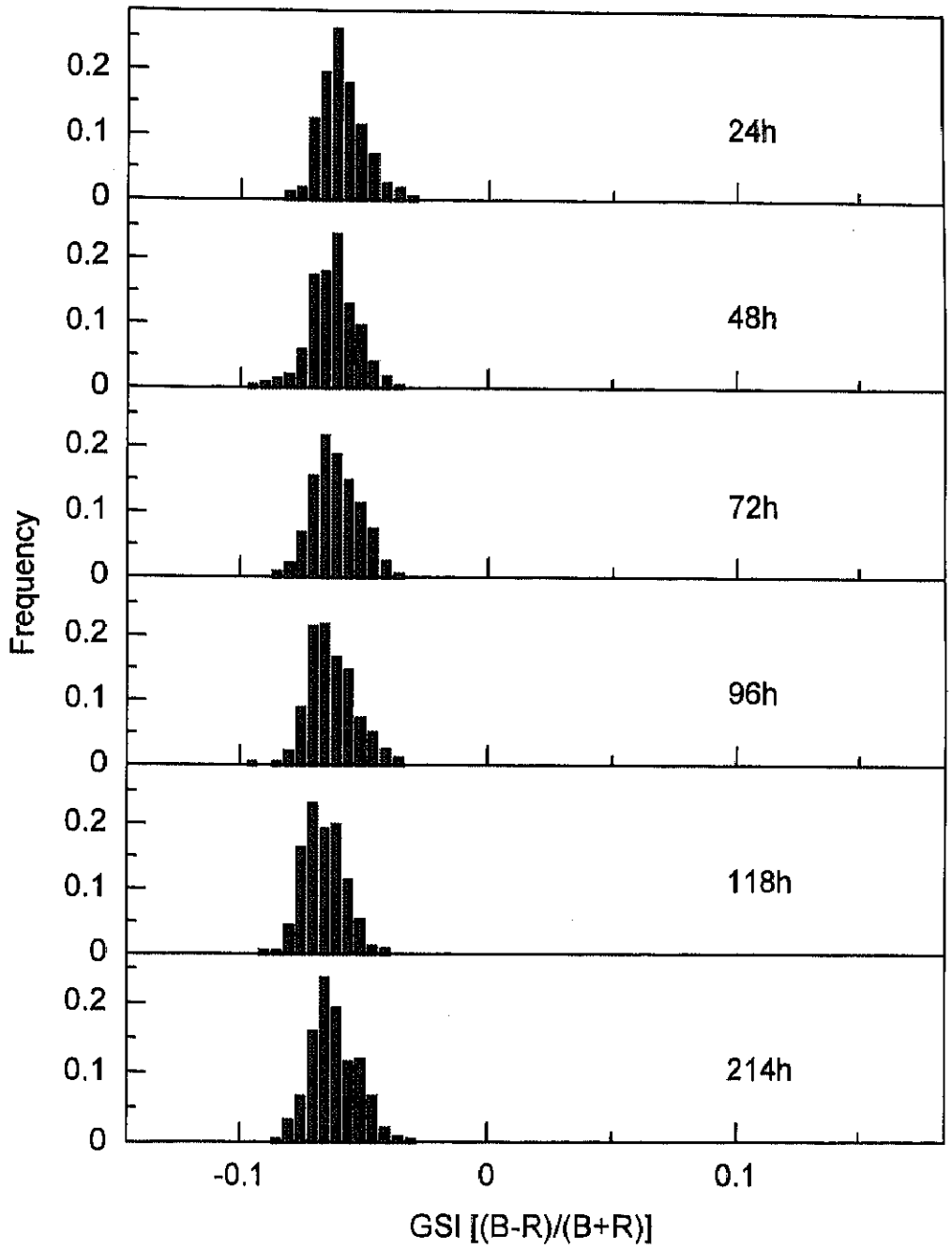

Fig. 5-3. GSI profiles of *H. salinarum* at different growth phases