Short Paper

Effect of Picoplankton *Synechococcus* sp. on the Growth and Survival of *Listonella anguillarum* (*Vibrio anguillarum*)Satoru Suzuki,*¹ Toshihide Suenobu,*¹ Hiroto Maeda,*²
and Riichi Kusuda*¹*¹Fish Disease Laboratory, Department of Aquaculture, Kochi University,
Nankoku, Kochi 783, Japan*²Lake Biwa Research Institute, Ohtsu, Shiga 520, Japan

(Received May 12, 1995)

Key words: *Synechococcus*, picoplankton, *Listonella anguillarum*, *Vibrio anguillarum*, growth, survival

As reported in our previous paper, picoplankton *Synechococcus* sp. clone P isolated from Lake Biwa was thought to accelerate the occurrence of vibriosis in ayu *Plecoglossus altivelis*.¹⁾ One of the mechanisms of this phenomenon was found to be a direct effect of picoplankton on ayu. The attachment of *Listonella anguillarum* (*Vibrio anguillarum*) to the skin of ayu was promoted when the fish were reared with picoplankton clone P. As the next step to determine the role of picoplankton in the acceleration of vibriosis in ayu, the effect of picoplankton on the growth and survival of the causative bacterium, *L. anguillarum* was investigated.

The strains of picoplankton and *L. anguillarum* used in this study were the same as those used in the previous study.¹⁾ The growth of *L. anguillarum* cultured with picoplankton was examined by using minimal medium for *Vibrio*.²⁾ Precultured cells of *L. anguillarum* were suspended in 20 ml of the minimal medium to give a final concentration of 10^4 cells/ml, and 30 ml of picoplankton suspension was added. The final concentration of picoplankton cells was approximately 10^6 cells/ml. This culture was incubated at 25°C with agitation. Time course change in number of *L. anguillarum* cells was measured by the viable count method using a solid medium (pH 7.8) containing 5 g polypeptone, 1 g yeast extract, 1 g proteose peptone, 1 g fish meat extract, 20 g NaCl and 15 g agar per l. The effect of culture supernatant and water extractable materials of picoplankton cells on the growth of *L. anguillarum* was also examined. A 4-week culture of picoplankton was centrifuged to separate the supernatant from the cells. The supernatant was lyophilized, and the cell pellet was disrupted by sonication (50 W, 5 sec, 5 times). The supernatant from the pellet was then obtained by centrifugation at $10,000 \times g$ for 15 min, followed by lyophilization. This fraction was used as water extractable materials from cells. Nine mg each of the lyophilized culture supernatant and cellular water extractable fraction was added to 1 ml of distilled water. They were added to 50 ml of minimal medium, and 0.5 ml of 3×10^6 cells/ml of *L. anguillarum* was inoculated. To measure the number of viable cells of *L. anguillarum* when inoculated with picoplankton, *L. anguillarum*

was inoculated in CT medium¹⁾ or distilled water containing 7×10^5 cells of picoplankton/ml. The number of *L. anguillarum* cells was counted as described above over time.

The growth of *L. anguillarum* in the medium supplemented with picoplankton clone P or G is shown in Fig. 1. Growth enhancement was observed in *L. anguillarum* cultures containing picoplankton, especially clone P. As a preliminary study to clarify the substance(s) affecting the growth of *L. anguillarum*, fractions of culture supernatant and cellular water extractable materials were added to the culture of *L. anguillarum*. The culture supernatant fraction did not enhance the growth (Fig. 2A), whereas cellular water extractable materials, either from clone G or P, enhanced bacterial growth (Fig. 2B). It is already known that *L. anguillarum* rapidly becomes non-culturable in

Fig. 1. Effect of picoplankton on the growth of *Listonella anguillarum*. Cultures with picoplankton clone P (□), clone G (△) or without picoplankton (○) are shown.

Fig. 2. Effect of culture supernatant (A) and cellular water extractable materials (B) of picoplankton on the growth of *Listonella anguillarum*.

Added fractions were supernatants of clone P (□) or clone G (△), and water extractable materials of clone P (■) or clone G (▲). The control (○) was not supplemented with any fractions.

fresh water.^{3,4)} Survival studies of *L. anguillarum* in CT medium and in distilled water showed a rapid decrease of the number of culturable cells of *L. anguillarum* within 1 h after inoculation. This suggests that coexistence of picoplankton does not give effect to elongate the survival period of *L. anguillarum*.

In this study, it was found that picoplankton, especially clone P, plays an important role in the growth enhancement of *L. anguillarum* although the factor(s) was not identified. This fact reminds us that the effect of picoplankton on the growth of *L. anguillarum* helps picoplankton clone P to accelerate vibriosis in ayu in the lake.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Fisheries Agency, Ministry of Agriculture, Forestry, and Fisheries of Japan.

References

- 1) S. Suzuki, T. Suenobu, H. Maeda, and R. Kusuda: *Fisheries Sci.*, **60**, 713-716 (1994).
- 2) S. Yamamoto, N. Okujo, Y. Fujita, M. Saito, T. Yoshida, and S. Shinoda: *J. Biochem.*, **113**, 538-544 (1993).
- 3) K. A. Hoff: *Appl. environ. Microbiol.*, **55**, 1775-1786 (1989).
- 4) T. Itami and R. Kusuda: *J. Shimonoseki Univ. Fish.*, **32**, 33-39 (1984).