ENVIRONMENTAL SCIENCES Vol. 1, No. 2, 99~103
Bulletin of the Korean Environmental Sciences Society

Comparison in Restriction Profile Analysis of *Vibrio furnissi*, *Vibrio fluvialis*, and *Vibrio parahaemolyticus* Bacteriophage from Sea Product

Younghee Kim

Department of Microbiology, Dongeui University,
Pusanjin-gu Gaya-dong, Pusan, 614-714, Korea

(Manuscript received on 18 September, 1997)

The bacteriophages lytic for *Vibrio furnissi*, *Vibrio fluvialis* and *Vibrio parahaemolyticus* were isolated from fish gills and shellfish. Nucleic acid of bacteriophage was prepared and restriction endonuclease profile was compared. All isolates contained deoxyribonucleic acid. *V. furnissi* bacteriophage from fish gills showed 2 bands with *Bgl* II, 1 with *Pst*, 3 with *Hind* III, 1 with *Bam* HI and 2 with *Eco*R I. *V. fluvialis* phage represented 7 fragments with *Bgl* II, 1 with *Pst*, 4 with *Hind* III, and 2 with *Eco*R I. *V. parahaemolyticus* produced 13 sites with *Hind* III and 4 sites with *Eco*R I. The fragment types were varied depending on the phage isolation. All three phages were digested with *Hind* III and *Eco*R I with different sizes. *V. furnissi* phage were digested with 5 different restriction enzymes.

Key words: Bacteriophage, *Vibrio furnissi*, *Vibrio fluvialis*, *Vibrio parahaemolyticus*, Deoxyribonucleic acid, *Pst*, *Bam* HI, *Hind* III, *Eco*R I, *Bgl* II.

1. Introduction

Vibrio is a natural habitant of a marine environment, its distribution is worldwide and many are already known pathogens to man and mariculture (Munro, 1995). Vibriosis has been estimated to cause great economic losses in mariculture. Vibriosis in bacterial fish pathogen may pose a potential public health hazard, since vibriosis from animals may be transferred to humans either directly, by infection with pathogens or indirectly, if they are transferred to human pathogens such as *V. cholera*, or *E. coli* by way of pathogenic fish bacteria. The populations of vibrio within the gut of estuarine animals are consistently high and is dependant on environment and their density varies with environment. Interposed within the gut populations of vibrosis is an equally abundant and apparently diverse populations of vibrio bacteriophages and these were consistently isolated from several sources due to contamination of host.

Attention to this is necesssary because these vibrios are shuttle bacteria between human and marine environment. Studies of bacteriophages have shown how to control the host (Yamamoto, 1970). The occurrence and distribution of bacteriophage (Adams, 1959) in estuarine environment is serious and the number and species of bacteriophage occur continuously in marine environment resulting from tidal periodicities, seasonal fluctuations in water temperature, dissolved-oxygen levels, and concentrations of organic nutrients from inland. (Hahn, 1991, Hong, 1994).

Genomic analysis can be a helpful tool to identify suspected pathogens, but not much is known about the characteristics of genomic traits of vibrio bacteriophages on the basis of its

molecular level. Restriction fragment length polymorphism (RFLP) has been used (Olsen, 1990, Rochelle, 1985, Elmerdahl and Larsen, 1990) in epidemiological studies to differentiate the bacteriophage (Allet *et. al.*, 1973, Bancroft and Freifelder, 1970).

To control and understand the disease the relationship between the host and parasite has to be studied, and an application of bacteriophage in bacteriological control in environment can be a host control pilot. Until now vibrio bacteriophages concerning morphological and physiological informations have been discussed, but no available report has been found on genomic analysis. And there is also the possibility that isolated bacteriophages may have biological differences with different geographical origins; continuous work is required to find out biological changes in these species.

Therefore the main purpose of this study is to compare and evaluate the restriction endonuclease profile analysis of vibrio bacteriophages and to obtain some results of genomic differences among species.

2. Materials and Methods

2.1. Host strains

Vibrio furnissi ATCC 35061, *Vibrio fluvialis* ATCC 33803, *Vibrio parahaemolyticus* O5:K57 were used for host strains for phage isolation. Nutrient broth containing 2% maltose and 3% NaCl with adjusted pH 7.6 were used for all host cell growth.

2.2. Bacteriophage isolation

Fresh fish gills and shellfish were purchased from commercial market and delivered to the laboratory as soon as possible in an ice container and used in an hour. Fish gills were chopped with a sterilized knife and minced in a mortar. Shellfishes were blended in a blender. Prepared samples were combined with a mixture of overnight grown cells of host and incubated for 48 hrs at 37 ℃. Centrifugation (12,000 x g, 10 min) was followed and the supernatant was harvested, filtered with 0.22 ㎛ membrane filter. This filtrate was mixed with same volume of soft agar containing Bacto beef extract 3 g, peptone 5 g, NaCl 3%, agar 0.7%, pH 7.2 at 50 ℃ and overlaid on the bottom agar which was composed of soluble starch 5 g, yeast extract 3 g, peptone 5 g, 0.7% agar, 3% NaCl and final pH 7.2. by using Adam's double agar overlaying method(1957). Bacteriophages were selected which formed clear plaques on lawns of their respective host strains were picked. The initial isolation of bacteriophage for the preparations of stocks involved the stabbing of a single plaque with a wire loop, which was then inoculated into 10 ㎖ of phage broth containing an early log phase culture of the host bacterial strain. A large amount of phage stock was obtained by repeating soft agar technique. It was kept in the refrigerator until use after addition of a couple of drops of chloroform.

2.3. Phage nucleic acid preparation

Phage nucleic acid was extracted by phenol and ethanol precipitation (Maniatis, 1982). It was treated with deoxyribonuclease and ribonuclease overnight at 37℃ waterbath.

2.4. Restriction enzyme analysis

Isolated phage nucleic acid was digested with commercially available restriction enzymes according to the supplier's instruction. Adopted restric-

tion enzymes were *Eco*R I, *Bam* HI, *Pst*, *Hind* III, and *Bgl* II.

2.5. Agarose gel electrophoresis

Electrophoresis in 0.8% agarose gels were run at a current of 3 v/cm in 1 x TAE buffer (Kim, 1996). Gels were stained in 2 mg of ethidium bromide per liter and photographed with a polaroid 667 film after the bands were detected under a UV illuminator.

3. Results and Discussion

The occurrence of marine vibrio bacteriophages were determined by inoculation of log phase cultures of different species of vibrios with various marine environmental mollusks. The presence and absence of bacteriophage varied from samplings. A high isolation of virus from fish gill was noticeable in this study; and mollusks also contained large amount of viruses. Host specificity was peculiar. We also examined bacteriophage from *V. anguillarium*, no phage was shown. Among the raw samples that we tested, phage from fish gill was specific for *V. furnissi*. Oysters and clams were a good source of *V. parahaemolyticus* and *V. fluvialis* phage isolation.

It is a matter of interest how the restriction types have evolved from each other and studies on the genetic relationships between the restriction enzyme profiles must be performed. The use of these profiles may facilitate the differentiation of vibrio bacteriophages. In this report we show that the bacteriophages digested with the restriction enzymes of *Eco*R I, *Bam* HI, *Pst*, *Bgl* II, and *Hind* III. The results are shown in Fig. 1.

All isolated bacteriophages contained deoxyribonucleic acid. Genomic analysis with restriction endonucleases digestion represented different features, showing 2 sites with *Bgl* II, 1 site with *Pst*, 3 sites with *Hind* III, 1 site with *Bam* HI and 2 sites with *Eco*R I, in *V. furnissi* phage originated from fish gills, 7 sites with *Bgl* II, 1 site with *Pst*, 4 sites with *Hind* III, and 2 sites with *Eco*R I in *V. fluvialis* phage obtained from oyster, and in *V. parahaemolyticus* phage isolated

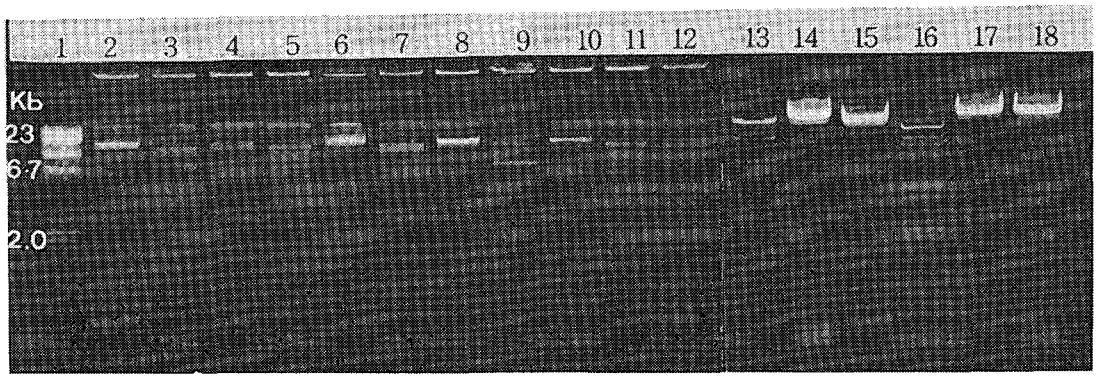

Fig. 1. Demonstration of restriction endonuclease cleavage patterns in vibrio phages. Lanes: 1, phage lambda cut with *Hind* III; 2-7, *V. furnissi* phage control, *Bgl* II, *Pst*, *Hind* III, *Bam* HI, *Eco*R I; 8-12, *V. fluvialis* phage control, *Bgl* II, *Pst*, *Hind* III, *Eco*R I; 13-18, phage lambda marker, *V. parahaemolyticus* phage control, *Eco*R I, *Hind* III, *Pst*, *Bam* H1 digestion.

from clam showed 13 sites with *Hind* III and 4 sites with *Eco*R I. The restriction enzymes of *Eco*R I and *Hind* III had sites for three different viruses. It was noticeable that *Eco* RI and *Hind* III sites were observed in three viruses and no fragment was shown in *V. parahaemolyticus* with *Pst* and *Bam* HI digestion. The result of *V. parahaemolyticus* was fully consistent with previous work (Kim, 1996) even though the virus origination was different.

It is possible that restriction endonuclease analysis can be used to satisfy discrimination among the vibrio phages and it can therefore be considered for use in genomic analysis. Continuous work with bacteriophages isolation will be required on the basis of diverse marine source samplings to evaluate fecal contamination in marine environment.

4. Conclusion

Isolation of bacteriophages from sea product were attempted to evaluate as an indicator of fecal contamination in marine environment. The bacteriophages lytic for *Vibrio furnissi, Vibrio fluvialis* and *Vibrio parahaemolyticus* were isolated from fish gills and shellfish. The isolated bacteriophages contained deoxyribonucleic acid and restriction endonucleases were applied to analyze their genomic differences. *V. furnissi* bacteriophage contained 2 sites with *Bgl* II, 1 site with *Pst*, 3 sites with *Hind* III, 1 site with *Bam* HI, and 2 sites with *Eco*R I digestion. *V. fluvialis* bacteriophage represented 7 sites with *Bgl* II, 1 site with *Pst*, 4 sites with *Hind* III, and 2 sites with *Eco*R I digestion. *V. parahaemolyticus* bacteriophage produced 13 sites with *Hind* III and 4 sites with *Eco*R I digestion. Restriction enzymes of *Eco*R I and *Hind* III digested three different bacteriophages and *V. furnissi* bacteriophage were digested with five different restriction enzymes.

Acknowledgements

This work was supported in part by the Institute for Biological-Production Research Center (IBP) of Dongeui Univ., 1997.

References

Adams, M. H., 1959, Bacteriophages, Interscience Publication Inc., New York.

Allet, B., P. G. N. Jeppesen, K. J. Katagiri and H. Delius, 1973, Mapping the DNA fragments produced by cleavage of lambda DNA with endonuclease R 1, *Nature*, 241, 120-123.

Bancroft, F. C and D. Freifelder, 1970, Molecular weights of coliphage and coliphage DNA: 1. Measurements of the molecular weights of bacteriophage T7 by high speed equilibrium centrifugation, *J. Mol. Biol.*, 54, 537-546.

Elmerdahl, J. O and J. L. Larsen, 1990, Restriction fragment length polymorphism of the *Vibrio anguillarum* serovar 01 virulence plasmid, *Appl. Environ. Microbiol.*, 56(10), 3130-3132.

Hahn, D., R. Margaret and A. Mchenny, 1991, Properties of the Streptomycete temperate bacteriophage FP43, *Journal of Bacteriology*, 173(12), 3770-3775.

Hong, S. E., N. Y. Cho G. Jeong, 1994, Isolation and partial characterization of a new *Escherichia coli* bacteriophage E3, *Kor. J. of Microbiol.*, 32(6), 464-470.

Kim, Y. H., 1996, Isolation and partial characterization of bacteriophage from oyster, *J. of the Korean Environmental Sciences Society*, 5(5), 605-610.

Maniatis, T., E. F. Fritsch and J. Sambrook, 1982, Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Munro, P. D., A. Barbour and T. H. Birkbeck, 1995, Comparison of the growth and survival of larval turbot in the absence of culturable bacteria with those in the presence of *Vibrio anguillarum, V. alginolyticus* or a marine *Aeromonas* sp., *Appl. Environ. Microbiol.*, 61(12), 4425-4428.

Olsen, J. E., 1990, An improved method for rapid isolation of plasmid DNA from wild-type Gram-negative bacteria for plasmid restriction profile analysis, *Lett. Appl. Microbiol.*. 10. 209-212.

Rochelle, P. A., J. C. Fry, M. J. Day and M. J. Bale, 1985, An accurate method for estimating sizes of small and large plasmids and DNA fragments by gel electrophoresis, *J. Gen. Microbiol.*, 132, 53-59.

Yamamoto, K. R., B. M. Alberts, R. Benzinger and L. Lawhore, 1970, Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large scale virus purification, *Virology*, 40, 734-744.