Mem. Fac. Fish., Kagoshima Univ.
Vol. 27, No. 1, pp. 59~64 (1978)

Studies on the Characteristics of the Bacteriophages of *Vibrio alginolyticus* strain B-1 Isolated from Kinko Bay

Kunihiko NAKAMURA,[*1] Daiichi KAKIMOTO,[*2]
James SWAFFORD,[*3] and Roy JOHNSON[*3]

Abstract

Eight variant bacteriophages infectious for *Vibrio alginolyticus* strain B-1, isolated from Kinko Bay, were isolated and purified. The virion consisted of an icosahedral head of 700 A, tail length of 1400 A and large plate. These bacteriophages were distinguishable reciprocally on plaque morphology, burst size, heat sensitivity, and the quality of resistance against host bacteria. These bacteriophages seemed to be naturally occurring variants produced in a variable environment of the seashore.

Hosaka[1] isolated and showed the morphology of *Vibrio parahaemolyticus* bacteriophages. Hori and co-workers[2] isolated the bacteriophages of *V. parahaemolyticus* from sea shores, stools of patients, and marine fish. Baross *et al.*[3] demonstated that shellfish which were shown invariably to harbor high titers of specific *V. parahaemolyticus* bacteriophages had the ability to transduce the agar hydrolyzing characteristic to *V. parahemolyticus* under simulated in condition in aquarium oyster.

Kakimoto and Nagatomi[4] isolated *Vibrio* strain B-1 bacteriophage from Kinko bay. Nakamura *et al.*[5] identified this Vibrio strain as *Vibrio alginolyticus* strain B-1. The phage makes various type of plaques on an agar plate in spite of repeated single plaque isolations. The authors tried to purify these phages and examine the characteristics of these *V. alginolyticus* strain B-1 bacteriophages.

Materials and Methods

Bacterial strains: The host strain was isolated by Kakimoto and Nagatomi[4] from Kinko Bay in 1970. Kakimoto *et al.*[5] calculated the similarity index between this strain and *V. parahaemolyticus* STO-5 for about 100 traits, including morphological, physiological, and biochemical observations and reported that this strain was a biotype of *V. parahaemolyticus*. Nakamura *et al.*[6] identified this

*1 Department of Bacteriology, Faculty of Medicine, Kagoshima University, Kagoshima 890.
*2 Laboratory of Microbiology, Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarata Kagoshima.
*3 Department of Botany and Microbiology, Arizona State University. Tempe, Arizona 85281, U. S. A..

strain as *Vibrio alginolyticus*. Authentic strains of *V. alginolyticus* 370, 5556, and 138-2 were obtained from Dr. Shimizu at the Unniversity of Tokyo. *V. parahaemolyticus* STO-5 was obtained from Dr. Sakai at Hokkaido University. *V. cholerae* ATCC 145035 was obtained from American Type Culture Collection.

Medium: The medium routinely used was made by dissolving the following components in one liter of distilled water; NaCl 30 g; KCl 0.7 g; $MgCl_2$ $6H_2O$ 10.8 g; $MgSO_4$ $7H_2O$ 5.4 g; $CaCl_2$ $2H_2O$ 1.0 g; yeast extract 1.0 g; polypeptone 5.0 g (ZoBell 2216 E Medium).

Purification of Bacteriophages: The phage lysates of *V. alginolyticus* strain B-1 had been purified from the phage lysate which was preparated by Kakimoto and Nagatomi[4], by the double layer method and were maintained at 15 C. Accordingly, the phages isolated from the centers of plaques with an inoculating needle were incubated with host bacteria at 37 C for 5 hrs and chloroform was added in the proportion of 1 to 10.

Then 0.5 % of soft agar containing both supernatant of the chloroform-treated lysate and host bacteria were poured onto plates. Plaques of this phage tend to deformed on moist agar plate and distinct plaques were difficult to obtain. Therefore, plates were dried at 37 C overnight before use. These techniques were repeated many times for additional phage types and subsequent purification depended on plaque morphology.

Isolated bacteriophages were examined using 10 min exposures to room temperatures, 60 C, 70 C, and 80 C. Heat sensitivity at 60 C was carried out by sampling at 10 min intervals for 60 min. Sensitivity of the phages to saturated chloroform, 1 % phenol and 0.25 % formaldehyde was also examined.

Plaques were stained by the method of JACKSON[7] and photographed for comparative morphology. Bacteriophage samples for electron microscopic observation were prepared by centrifuging for 3 hrs at 35000 r.p.m. on Beckman Model E ultracentrifuge. The bacteriophage pellet was washed twice in 1 % ammonium acetate, pH 7.0. Lysates were placed on 300 mesh copper grids supported with carbon-stabilized formvar and negatively stained with 1 % sodium phosphotungstate, pH 7.0.

Results and Discussion

Eight bacteriophages infectious for *V. alginolyticus* strain B-1 were isolated and purified from a lysate of *V. alginolyticus* strain B-1. Fig. 1 shows the diverse plaque morphology of these phages. No morphological difference was noted among these bacteiophages. The typical shape of these bacteriophages is shown in Fig. 2. The virion consists of an icosahedral head of 700 A, a tail length of 1400 A and a large tail plate. The shape and size of this virion were identical with those infectious for *V. parahaemolyticus* strains reported by Sklarow *et al.*[8]. All these were uniformly inactive on *V. parahaemolyticus* STO-5, *V. alginolyticus* 370, 5556, 138-2 and *V. cholerae* ATCC 14035.

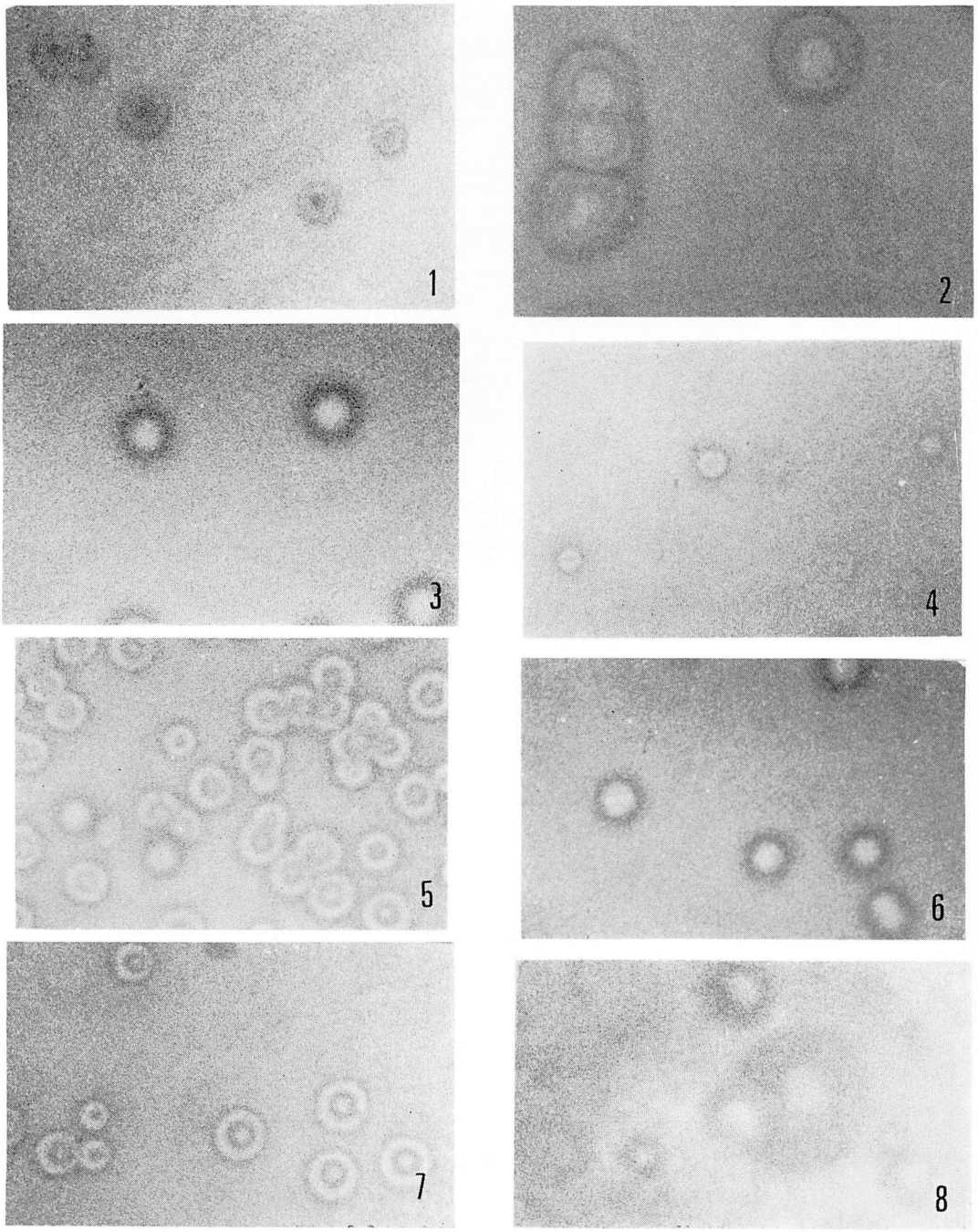

Fig. 1. Plaque morphology of bacteriophages of *V. alginolyticus* strain B-1. Stained by the method of Jackson. Abbreviations indicate:
1···a phage; 2···b phage; 3···0 phage; 4···m phage; 5···h phage; 6···x_4 phage; 7···x_7 phage; 8···x_9 phage.

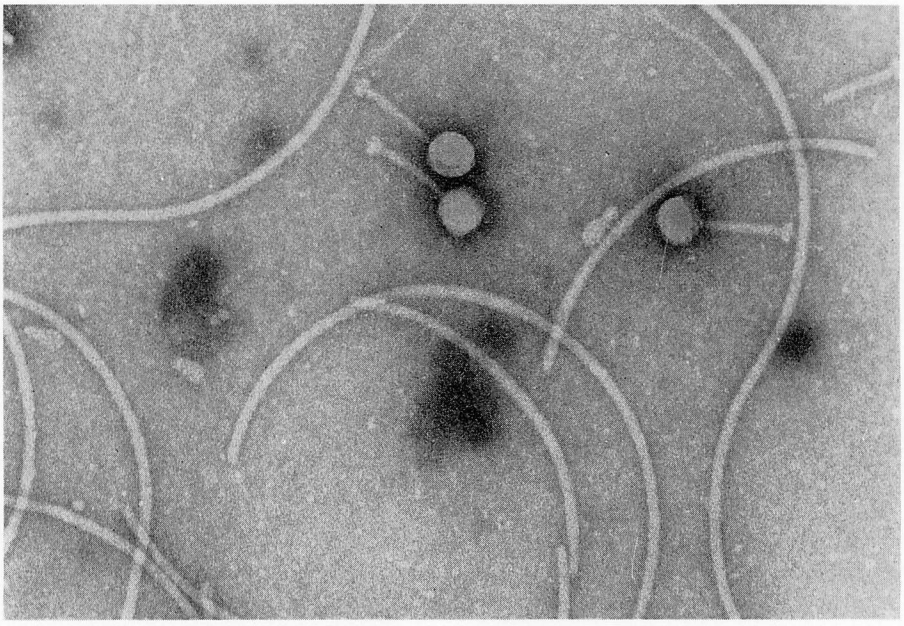

Fig. 2. The morphology of *V. alginolyticus* strain B-1 bacteriophage particle negatively stained. × 30000

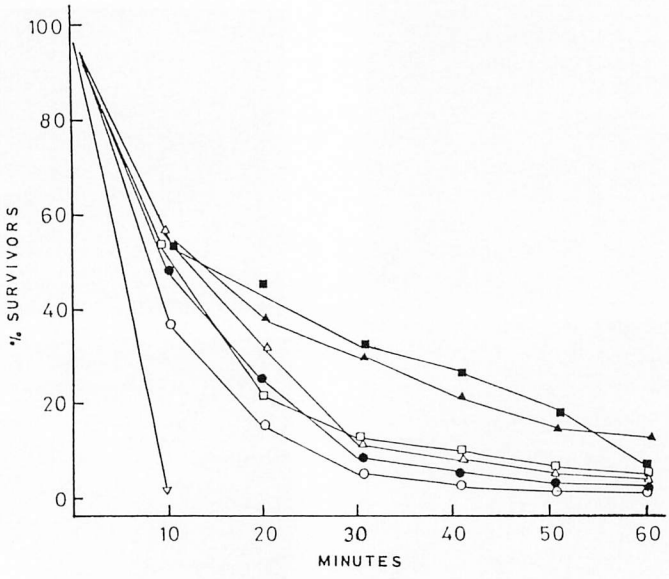

Fig. 3. The heat inactivation patterns of *V. alginolyticus* strain B-1 bacteriophages at 60 C.
a···△; b···●; m···□; 0···■; h···▲; x_7···○; x_4 x_9···▽.

Many phage-resistant strains were isolated and tested for their sensitivities to the other seven phages (Table 1). All eight phages were resistant to satur-

Table 1. Cross infectivity pattern of phage strains on isolated resistant strains of *V. alginolyticus* strain B-1.

Resistant Bacterial Strains	Phage strains							
	a	b	o	m	h	X_4	X_7	X_9
R_1	⫲*	⫲	⫲	⫲	⫲	⫲	⫲	+
R_2	⫲	⫲	⫲	⫲	⫲	⫲	+	⫲
R_3	⫲	⫲	⫲	+	⫲	⫲	⫲	⫲
R_4	−	−	−	−	−	−	−	−
R_5	⫲	⫲	⫲	⫲	⫲	⫲	⫲	⫲
R_6	⫲	⫲	⫲	⫲	⫲	+	+	⫲
R_7	⫲	⫲	+	+	+	+	+	+
R_8	⫲	⫲	⫲	⫲	+	+	⫲	⫲
R_9	+	+	+	+	+	+	+	+
R_{10}	⫲	⫲	+	+	⫲	+	+	⫲
R_{11}	⫲	⫲	+	⫲	⫲	⫲	+	⫲
R_{12}	+	−	−	−	+	+	−	⫲
R_{13}	⫲	+	+	+	+	+	+	⫲

* ⫲ highly sensitive + weekly sensitive
⫲ moderately sensitive − resitant

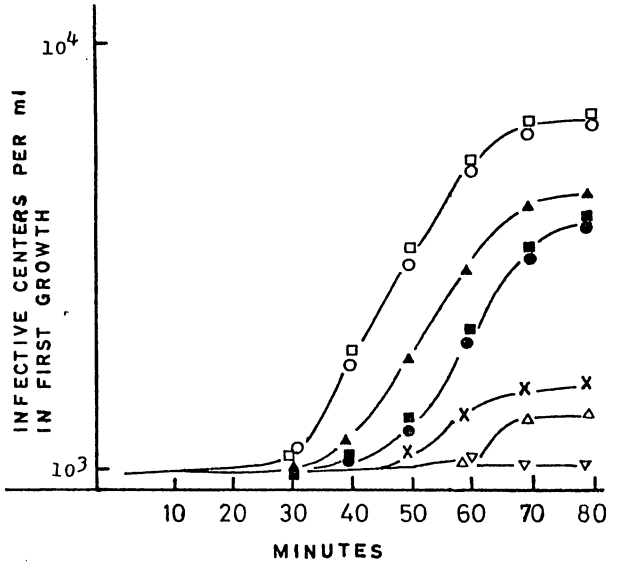

Fig. 4. One step growth curves of *V. alginolyticus* strain B-1 phages at 37C.

a···△; b···●; m···□; 0···■; h···▲; x_4···▽; x_7···○; x_9···×.

ated chloroform and sensitive to both 1% phenol and 0.25% formaldehyde. All of the bacteriophages used for heat sensitivity measurment were inactivated at 70 C for 10 min. Fig. 3 shows the heat inactivation time of these bacteriopha-

ges at 60 C. Phages X_4 and X_9 were inactivated significantly more rapidly than the other phages. The burst sizes of the phages are shown in Fig. 4. From these observations, phages isolated were presumed to be dissimilar because of their plaque morphology, heat sensitivity and burst size.

In spite of isolating from the center of the plaque with an inoculating needle, almost these bacteriophage variants except for original phage occurred naturally during purification. For this reason, the enrichment process of the phages had to be done in a short time incubation. The numbers of the morphologically same plaques were increased depending upon short time enrichment process and many times purification. However we couldn't get really purified bacteriophages of strain B-1.

The variability of these phages may depend on both short generation time of the host bacteria and lysogenecity of these bacteriophages.

Acknowledgements

K. Nakamura in master course studies of Faculty of Fisheries has performed at Arizona State University under the patronage of the student exchange program of the Japanese education administration. We are thankful to Mr. Uehara, for his skilled and conscientious aid in electron microscopic observation at Kagoshima University.

References

1) HOSAKA, Y. (1966): The morphology of V in phage 14. Electron micrographs biology-2 Hitachi Electron Microscope.
2) HORI, M., K. MAESHIMA, S. NAKANISHI, Y. IIDA and T. TERAMOTO. (1967): Studies on bacteriophages of *Vibrio parahaemolyticus*. "*Vibrio parahaemolyticus*" **2**, 147-165.
3) BAROSS, J. A., J. LISTON and R. Y. MORITA. (1974): Some implications of genetic exchange among marine vibrios, including *Vibrio parahaemolyticus*, naturally occurring in the Pacific oyster. "International symposium on *Vibrio parahaemolyticus*" 129-137.
4) KAKIMOTO, D., and H. NADATOMI. (1971): Study of bacteriophages in the Kinko Bay. *Bull. Jap. Soc. Fish.* **38**, 271-278.
5) KAKIMOTO, D., K. NAKAMURA, and Y. TANAKA. (1972): Studies on the vibrios and pseudomonads living in the Kinko Bay. *Mem. Fac. Fish. Kagoshima. Unive.* **21**, 109-112.
6) NAKAMURA, K., D. KAKIMOTO, J. SWAFFORD and R. JOHNSON. (1979): in press.
7) JACKSON, R. H. (1967): Differential staining of bacteriophage plaques for photograph. *Can. J. Microbiol.*, **13**, 117-118.
8) SKLAROW, S. S., R. R. COLWELL, G. B. CHAPMAN and S. ZANE (1973): Characteristics of a *Vibrio parahaemolyticus* bacteriophage isolated from Atlantic coast sediment. *Can. J. Microbiol.*, **19**, 1519-1520.