九州大学学術情報リポジトリ
Kyushu University Institutional Repository

Bacteriophages of Bacillus natto : 1. Some characteristics of phage NP-1

Yoshimoto, Akihiro
Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Kyushu University

Hongo, Motoyoshi
Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Kyushu University

https://doi.org/10.5109/22801

出版情報：九州大学大学院農学研究院紀要. 16 (2), pp.141-158, 1970-07. Kyushu University
バージョン：
権利関係：

Journal of the Faculty of Agriculture, Kyushu University, Vol. 16, No. 2
July 31, 1970

Bacteriophages of *Bacillus natto*

1. Some characteristics of phage NP-1

Akihiro YOSHIMOTO[1] and Motoyoshi HONGO[1]

Bacillus subtilis phages were first reported by Fukuda[10] and subsequently by Kitahara et al.[13] and Watanabe et al.[34] as phage active against amylase-producing strains of *B. subtilis*. Kinoshita et al.[12] also isolated a *subtilis* phage from seweage. Afterward, as a result of discovery of DNA-mediated transformation, many phages which infected a transformable Marburg strain of *B. subtilis* have been actively isolated for the investigation of genetic phenomena. A series of phages SP were isolated by Romig et al.[23] and transducing phages were isolated by Takagi,[26] Takahashi[27] and Thorne et al.[31]

Recently, Brodetsky et al.[5] studied on various properties of their *subtilis* phages, set them apart from some other *subtilis* phages and distinguished them from each other. *Subtilis* phages have the notable characteristics that some of them contain unusual base such as uracil or hydroxymethyl uracil in their DNA[11,24,30] and most of the temperate phages can transduce various makers.[26,27,31] Therefore, with *Bacillus natto* phage, it would be also expected to find out such characteristics as observed with *subtilis* phages. A virulent phage of *B. natto* was isolated from abnormally fermented "natto" and characterized by Fujii et al.[9]

This paper presents some characteristics of a temperate phage which was isolated from abnormal "natto" forming no mucilage.

Materials and methods

Bacterial strains

Strain Takahashi No. 1 and No. 3 of *Bacillus natto* were obtained from Prof. H. Fujii, Fukuoka Women's University. Strain No. 3 was used as a host bacterium. *B. subtilis* var. *amyloliquefaciens* was obtained from

1) Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Kyushu University, Fukuoka, Japan

Prof. K. Watanabe, Fukuoka University and other *Bacillus* strains were obtained from Prof. Y. Ikeda, Tokyo University. *Escherichia coli* strain B was obtained from Prof. H. Endo, Kyushu University.

Phages

A phage of *B. natto* was isolated from abnormal "natto" forming no mucilage by usual technique and purified twice by single plaque isolation. This phage was designated NP-1. Stock phages were prepared by centrifuging the phage lysate at 8000 rpm for 15 min, and the supernatant fluid was filtered throughout seizfilter and stored at 4°C. The phage stock with titers of 10^9 to 10^{10} plaque-forming units (PFU) per ml was routinely obtained. Phage suspension having high titer was prepared by suspending the phages collected by a differential centrifugation in buffered saline (0.1 M NaCl and 0.005 M $MgSO_4$ in 0.06 M phosphate buffer, pH 7.2).

Coli phages T_2, T_3 and T_5 were obtaind Prof. H. Endo. Phages were assayed according to the double layer method by Adams.[1] Physiological saline or buffered saline was used for the dilution of phages.

Media

PY medium used for the propagation of *Bacillus* strains and phage NP-1 contained 1 g glucose, 10 g peptone, 1 g yeast extract, 0.02 g $CaCl_2 \cdot 2H_2O$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.05g K_2HPO_4 and 3 g NaCl in 1,000 ml of distilled water, pH 7.0. For the propagation of *E. coli* strain B and *coli* phages, PY medium from which yeast extract was removed was used.

Preparation of antiphage serum

The antiserum of phage NP-1 was prepared according to the method of Adams.[1] The obtained antiserum had a neutralization rate constant (K) of about 60 min^{-1}.

Host range

Host range was determined by spot test; overnight cultures of various *Bacillus* strains grown in PY medium were overlaid on the basal layers, and one drop of phage stock was spotted on the plates. The plates were incubated at 37°C overnight, and the resulting lysis area was examined.

Adsorption

For the determination of adsorption rate 0.1 ml of phage suspension (10^{10} PFU/ml) was added to 10 ml of 4-hr incubated host culture. The mixture was shaken at 37°C, and 0.1 ml was removed at various time

intervals and was immediately diluted 1 : 100 with chilled saline. After centrifugation, the supernatant fluids was assayed for unadsorbed phages. Adsorption rate (K) was calculated according to Adams.[1]

Effect of various metal ions on the adsorption was tested in Tris buffer (0.01M, pH 7.0) containing each metal ion; host culture (4×10^8 viable cells/ml) was centrifuged and the cells were suspended in the original volume of Tris buffer containing 0.01M EDTA. The cell suspension was stood for 5 min with occasionally shaking. After centrifugation, the cells were washed twice with Tris buffer in the cold and finally suspended in the original volume of the same buffer containing each metal ion. To each 5 ml of the cell suspensions was added 0.1 ml of phage suspension (5×10^5 PFU/ml), and the mixtures were incubated at 37°C for 10 min with shaking. After centrifugation, the supernatant fluids were assayed for unadsorbed phages.

Thermal inactivation

For the determination of thermal inactivation, the tubes containing 4.5 ml of buffered saline were maintained at various temperatures, and 0.5 ml of phage suspension (10^{10}PFU/ml) was then added to them and throughly mixed. At various time intervals 1 ml was removed and added to 9 ml of chilled saline. Phage survivals were assayed after appropriate dilution.

UV inactivation

Ten ml of buffered saline containining phages (10^9 PFU/ml) in a petri dish (9 cm in diameter) was irradiated at a distance of 40 cm under 15-W Toshiba germicidal lamp GL-15, while the dish was gently swirled by hand. Samples taken at various time intervals were diluted and assayed for phage survivals.

Preparation on phage DNA

Thirty shaking flasks containing 100 ml of PY medium were inoculated with 1 ml of overnight culture and shaken at 37°C for 3 hr. The cultures were infected with phage NP-1 at a multiplicity of about 3 and incubated further for 4 hr. Although the cultures were not completely lysed by this time, they were centrifuged at 9,000 rpm for 15 min. The obtained supernatant fluid (2,800 ml) contained phages of 10^{10} PFU/ml. Phages were collected from the supernatant fluid by ultracentrifuge at $35,000\times g$ for 1 hr. The phages were suspended in 60 ml of buffered saline and again centrifuged at 9,000 rpm for 15 min. To the phage suspension were added RNase and DNase (Shigma Co.) to give a final concentration of each 20 μg/ml. The suspension was incubated at 37°C for 30 min. The phages were then purified by two

cycles of differential centrifugation. The phage pellet was suspended in 20 ml of buffered saline. Phage DNA was extracted according to the method by Mandel et al.[17] To the phage suspension was added an equal volume of phenol saturated with water and the mixture in a glass-stoppered flask was shaken by hand for 5 min. The resulting emulsion was separated by centrifugation at 4,000 rpm for 10 min. The aqueous layer was removed by pipetting out. This procedure was repeated three times. The obtained aqueous fraction made to be free from phenol by extracting three times with ether. Ether was removed under reduced pressure at 30°C. The aqueous solution was then dialyzed against standard saline citrate (1.5 M NaCl in 0.15 M citrate buffer, PH 7.2). DNA was estimated by the diphenyl amine method.[6] This preparation contained 680 μg/ml of DNA and was still contaminated with 1.5 % RNA and 1 % protein.

Base composition of phage DNA

Phage DNA was precipitated from the DNA solution with two volumes of 95 % ethanol and dried in vacuo. The DNA was hydrolyzed with formic acid (over 98 %) in a sealed glass tube at 175°C for 30 min. The hydrolyzed DNA solution was evaporated to dryness under reduced pressure. To the residue was added 1 ml of 1 N HCl. The solution was submitted to descending paper chromatography, using Whatman No. 1 filter paper and a solvent system of isopropanol-concd. HCl-water (15 : 17 : 18).[35] The spots on the chromatogram were detected under a Manasulu light (Manasulu Chemical Industries). The detected area were cut out, and the base in each area was eluted with 0.1 N HCl. The bases were determined spectrophotometrically with a Hitachi spectrophotometer (model EPU 2A) with use of the following molecular extinction coefficients at 260 mμ[33] : adenine, 12,700; guanine, 8,100; cytosine, 6,200; thymine, 7,400.

Determination of Tm of phage DNA

Tm value was determined by the method according to Marmur and Doty[18] with modification. The glass-stoppered tubes containing about 100 μg of DNA in 5 ml of 10-fold diluted saline citrate were heated at a rising rate of 1°C up per 2 min after the temperature was somewhat rapidly increased until 70°C. One tube was taken at about 2°C intervals, immediately chilled in ice water and the absorbance at 260 mμ of the sample was measured with a Hitachi spectrophotometer. Guanine plus cytosine contents were calculated according to Marmur and Doty's equation: [18]

$$\text{Per cent } G+C=(Tm-69.3)/0.41$$

Amino acid analysis of phage protein

Phage NP-1cl, a clear plaque mutant, was harvested from 2,000 ml of the phage lysate which contained about 8×10^9PFU/ml. The phages were washed by two cycles of differential centrifugation as described above. Phage pellet was suspended in M/80 Tris buffer (pH 7,2) containing 10^{-3}M $MgSO_4$ and applied to DEAE-cellulose column chromatography. The column (3.0×20 cm) was equilibrated with the same buffer. After the phage solution (8×10^{10}PFU/ml, 20 ml) was placed and then 30 ml of the same buffer was poured, the phage was eluted with a stepwise of NaCl concentration in the buffer. The fractions containing phage were collected and centrifuged at 48,000×g for one hour. After one cycle of differential centrifugation, the phage pellet was taken into a small glass tube and 1.5 ml of 6N HCl was added. The tube was sealed and heated for 20 hr in boiling water. The acid hydrolysate was repeatedly evaporated to remove HCl in adding a small volume of water. The residue was examined for amino acids by auto-amino acid analyzer (Hitachi).

Lysogeny

To determine the frequency of lysogenization 4-hr incubated culture (2×10^8 viable cells/ml) was infected with phage at various multiplicities at 37°C. After 5-min adsorption the culture was treated with antiserum (1: 50) for 10 min. The culture was diluted appropriately with saline and spreaded on PY agar plates. After the plates were incubated overnight, the resulting colonies were picked out on bouillon agar slant and transferred successively three times on the new slants. The last slant culture were tested for the phage prducibility and resistance.

Results and discussion

Stability

Phage lysate did not almost decrease in the viability for one month at 4°C. As shown in Table 1, the phage was considerably inactivated in the various concentration of phosphate buffer except for 0.01 M. The addition of 0.01 M divalent cations to the buffer stabilized the phage. However, Cu^{++} and Zn^{++} had no stabilizing effect. Some other solutions were also examined. The phage was stable in saline citrate buffer, buffered saline, physiological saline and Tris buffer. Chloroform inactivated the phage. Effect of pH on the phage stability was examined in the various pH of PY medium. The result is shown in Fig. I.

Table 1. Stability of phage in various buffer solutions.

Buffer solutions	Phage surviivals (%)
Phosphate buffer (pH 7.0—7.2)	
6×10^{-2}M	0
1×10^{-2}M	102
1×10^{-3}M	21
1×10^{-4}M	30
6×10^{-2}M+10^{-2}M $MgSO_4$	105
6×10^{-2}M+10^{-3}M $MgSO_4$	8
6×10^{-2}M+10^{-3}M $MgSO_4$+10^{-1}M NaCl	100
6×10^{-2}M+10^{-2}M $CaCl_2$	85
6×10^{-2}M+10^{-3}M $CaCl_2$	10
6×10^{-2}M+10^{-2}M $BaCl_2$	105
6×10^{-2}M+10^{-2}M $CdCl_2$	100
6×10^{-2}M+10^{-2}M $CoCl_2$	114
6×10^{-2}M+10^{-2}M $CuCl_2$	20
6×10^{-2}M+10^{-2}M $MnCl_2$	90
6×10^{-2}M+10^{-2}M $NiSO_4$	117
6×10^{-2}M+10^{-2}M $SrCl_2$	106
6×10^{-2}M+10^{-2}M $ZnCl_2$	3
Saline-citrate (pH 7.2)	
1.5M—1.5×10^{-1}M	94
1.5×10^{-1}M—1.5×10^{-2}M	16
Physiological saline (1.5×10^{-1}M)	99
Tris buffer (10^{-2}M, pH 7.2)	94
Water	4

Phage suspensions were incubated at 37°C for 2 hr.

Phage and plaque morphology

Electromicroscopic photograph of the phage is shown in Fig. 2a. The phage had a regular six-sided head and a long noncontractile tail with its terminal appendage. The head diameter was about 600×600 A°. The tail was about 80×1,800A° in size and had a forked tip. It was also mostly observed as a crooked form. The empty phage with its transparent had a hollow tail exhibiting the capsomer structure more clearly (Fig. 2b).

The typical plaques of phage NP-1 and its clear plaque mutant, NP-1 cl, are shown in Fig. 3. NP-1 formed a turbid plaque of 1.5 to 3 mm in a diameter with peripherical ring. NP-1 assay plate contained usually some smaller plaques (0.25 to 0.5 mm) and a clear plaque at a rate of

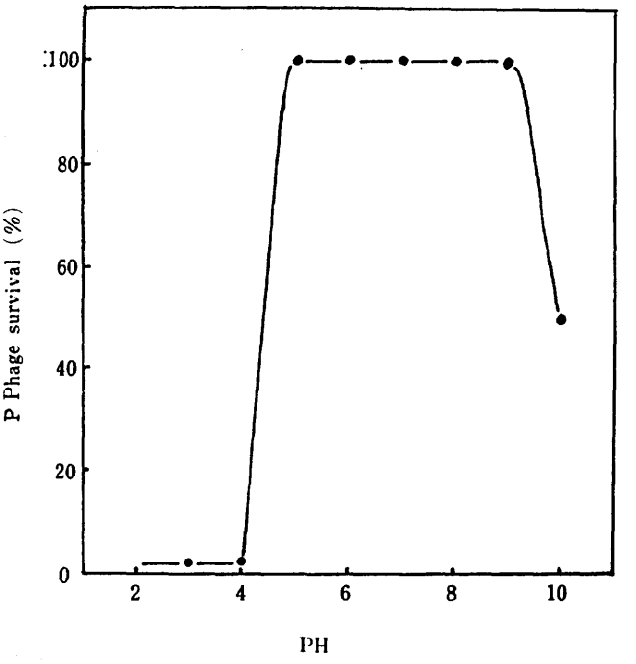

Fig. 1. pH stability of phage NP-1.
Incubation: 1 hr at 37°C

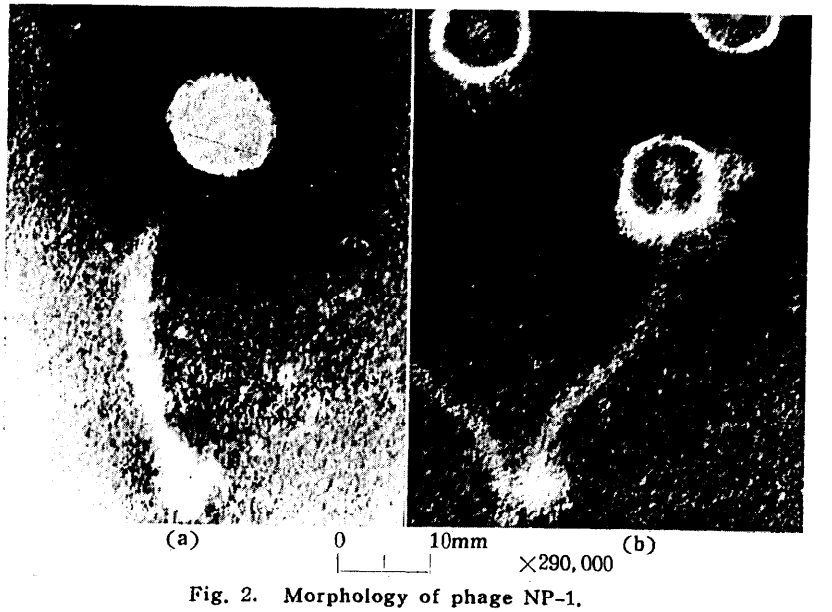

Fig. 2. Morphology of phage NP-1.

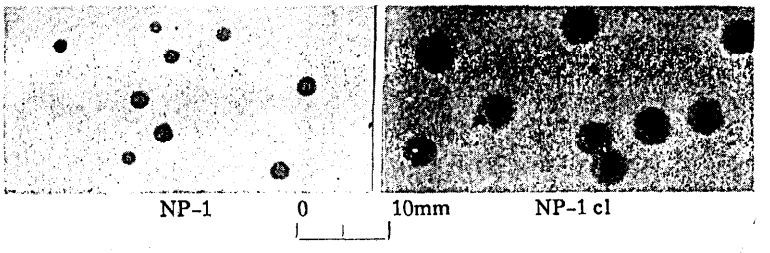

Fig. 3. Plaques of phage NP-1 and NP-1 cl.

about one per 1000 plaques. NP-1 cl formed more or less larger plaque than NP-1. Both plaques were surrounded by halo with a width of about 1.5 mm. Halo formation was observed with *Bacillus megaterium* phage,[19] *coli* phage T_2[3,14] and *Staphylococcus* phage.[22] Brodetsky et al.[5] also described that their *subtilis* phages formed halo.

Host range

Host range is shown in table 2. The phage was susceptible to *B. natto*, but not to any other *Bacillus* strains tested.

Table 2. Host range.

Organisms	Strains	Susceptibility to phage
Bacillus subtilis	Marburg, wild	—
Bacillus subtilis	Marburg, W 23	—
Bacillus subtilis	IAM 1523 (K)	—
Bacillus subtilis	amyloliquefaciens Fukumoto, K 49	—
Bacillus natto	Takahashi No. 1	+
Bacillus natto	Takahashi No. 3	+
Bacillus megaterium	203	—
Bacillus megaterium	899	—
Bacillus megaterium	IAM 1030	—
Bacillus megaterium	IAM 1032	—
Bacillus cereus	IAM 1029	—
Bacillus cereus	IAM 1072	—
Bacillus cereus	IAM 1229	—
Bacillus circulans	IAM 1112	—
Bacillus circulans	IAM 1140	—
Bacillus polymyxa	IAM 1189	—
Bacillus macerans	IAM 1227	—
Bacillus bervis	IAM 1031	—

Adsorption

The phage was adsorbed on host bacteria at a rate of about 95 % in 5 min. Adsorption rate constant (K) was 6×10^{-9}ml/min. The bacteria killed over 99 %, which were obtained by heating at 80°C for 10 min, also adsorbed about 80 % of input phage in 10 min. Table 3 shows the effect of various metal ions on the adsorption. The phage did not adsorbed to the bacteria suspended in Tris buffer without metal ion, but the addition of metal ion (Mg^{++}, Ca^{++}, Mn^{++}, Ni^{++}, Co^{++}, Na^{+} and K^{+}) to the bacterial suspension allowed to adsorb the phage.

Table. 3 Effect of various metal ions on adsorption of phage NP-1.

Metal ion	Concentration		
	10^{-1}M	10^{-2}M	10^{-3}M
		adsorption %	
Mg^{++}	—	98.2	94.0
Ca^{++}	—	98.0	86.5
Mn^{++}	—	97.7	83.3
Ni^{++}	—	98.9	95.0
Co^{++}	—	98.8	96.5
Na^{+}	99.2	95.0	58.0
K^{+}	—	96.4	52.4
None		4.0	

Growth characteristics

One-step growth experiments were carried out according to the method of Adams,[1] and latent periods and burst sizes were determined. Phage NP-1 had a burst size of 40 to 60 and NP-1cl had that of 80 to 120, but these values varied from experiment to experiment. Typical patterns were show in Fig. 4. Both the latent periods were about 40 min.

Table 4 shows the effect of divalent cation on the phage propagation. A high concentration of Mg^{++}(0.01 M), which was not necessarily required for the phage adsorption and the bacterial growth, was indispensable for the optimum propagation of the phage. The poor production at a low concentration of Mg^{++} may be attributable to both the smaller burst size and the abortive infection. Whereas, Ca^{++} was not effective although it is generally essential for the propagation of many kinds of phage. Mn^{++}, Ni^{++} and Co^{++} also had no effect.

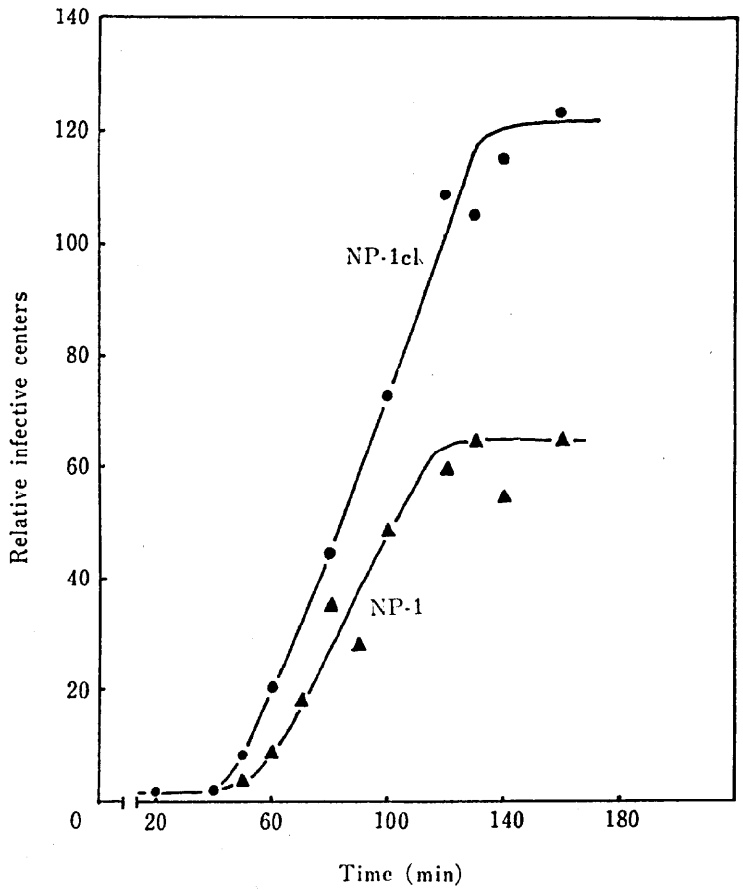

Fig. 4. One-step growth curves of phage NP-1 and NP-1cl.

Table 4. Effect of Mg^{++} and some other divalent cations on phage NP-cl propagation.

Metal ion added (M)	Time required for lysis (hr)	Final pH	Phage (PFU/ml)
Mg^{++} 5×10^{-2}	2.5	5.4	1.2×10^{10}
Mg^{++} 2×10^{-2}	3.5	5.4	2.3×10^{10}
Mg^{++} 1×10^{-2}	3.5	5.8	3.0×10^{10}
Mg^{++} 5×10^{-3}	3.5	6.0	2.6×10^{10}
Mg^{++} 2×10^{-3}	4.0	7.0	6.0×10^{9}
Mg^{++} 1×10^{-3}	4.0	5.8	2.0×10^{9}
Mg^{++} 1×10^{-4}	nonlysis	5.8	1.0×10^{8}

Metal ion added (M)	Time required for lysis (hr)	Final pH	Phage (PFU/ml)
Mg^{++} 1×10^{-3} supplimented			
with $Ca^{++}1\times10^{-2}$	4.0	6.8	9.0×10^{8}
〃 $Ca^{++}5\times10^{-3}$	4.0	6.0	1.0×10^{9}
〃 $Ca^{++}1\times10^{-3}$	4.0	5.8	2.0×10^{9}
〃 $Mn^{++}2\times10^{-3}$	4.0	5.8	1.0×10^{9}
〃 $Ni^{++}2\times10^{-3}$	4.0	5.8	8.5×10^{8}
〃 $Co^{++}2\times10^{-3}$	4.0	6.0	9.5×10^{8}

Host bacteria were grown in PY medium containing the indicated metal ion. When the cultures reached to 0.5 at OD 660 (about 2×10^{8} viable cells/ml), they were infected with phage at a multiplicity of 3 and incubated for 4.5 hr.

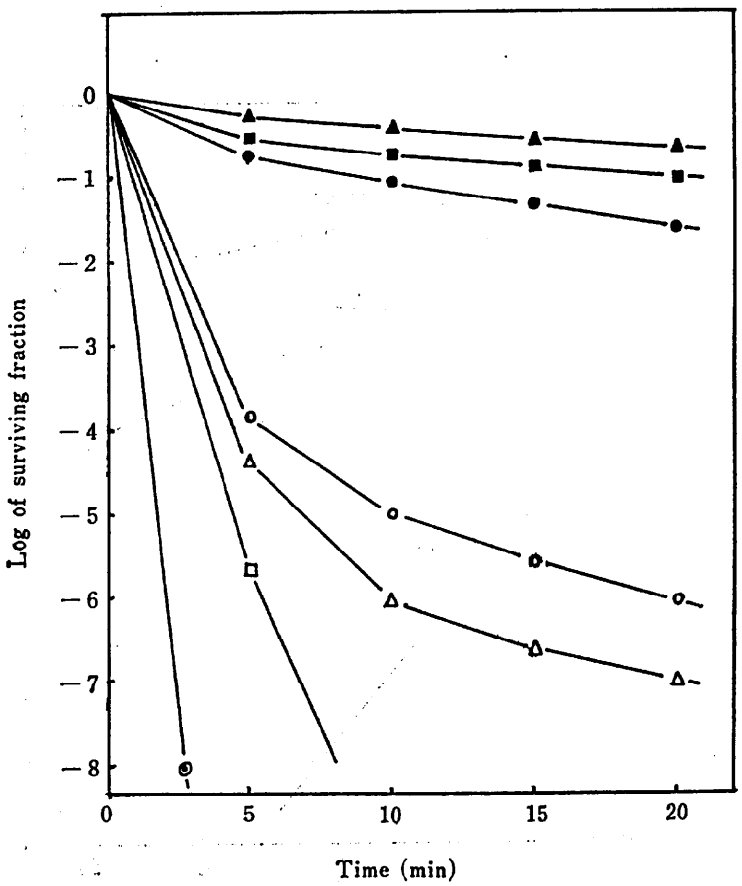

Fig. 5. Thermal inactivation of phage NP-1.
NP-1: 50°C (●), 60°C (○), 65°C (△), 70°C (□), 80°C (⊙)
T_2: 65°C (■); T_5: 65°C (▲).

Thermal inactivation

The phage was inactivated at 50°C and drastically at the temperature more than 60°C as shown in Fig. 5. Phage NP-1 was more sensitive to temperature than *coli* phage T_2 and T_5.

UV inactivation

The inactivation of phage by UV irradiation is shown in Fig. 6. The phage was strongly resistant to UV as compared with phage T_2 and T_3. The UV-inactivationcurve of NP-1 resembled that of phage SP 8.[5] Okubo et al.[20] and Mahler[16] described phage SPO 2 and SP 3 to be similarly very resistant to UV, and they established that the UV resistance was attributable to the host cell reactivation[10] by which UV-damaged phage DNA was repaired in the dark. The UV resistance of phage NP-1 may be dependent on the host cell reactivation.

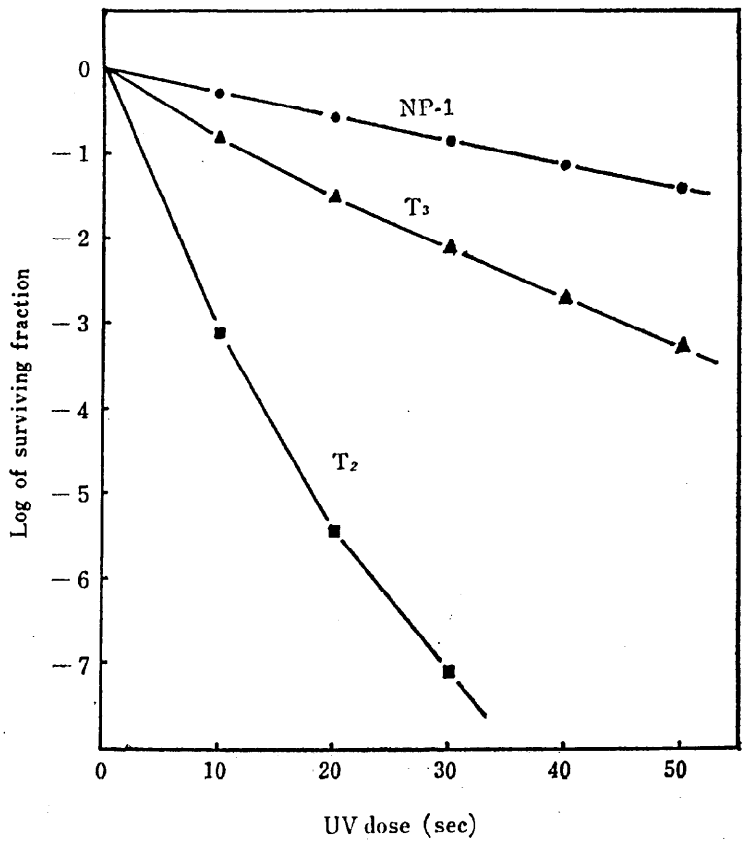

Fig. 6. Ultraviolet inactivation of phage NP-1.

Base composition of phage DNA

The base composition of phage DNA is shown in Table 5. The DNA contained no unusual base. The molar ratio of the bases was 1.00 : 0.69 : 0.71 : 1.02 with respect to adenine, guanine, cytosine and thymine in this order. Therefore, guanine plus cytosine contents were 40.9 %. The contents were also estimated to give 41.9 % from thermal denaturation temperature (Tm) of the DNA. The denaturation curve is shown in Fig. 7.

Table 5. Base composition of phage DNA.

Analytical methods	Bases	Molar ratio	G+C contents
Acid hydrolysis	Adenine	1.00	40.9 %
	Guanine	0.69	
	Cytosine	0.70	
	Thymine	1.01	
Tm		(85.6°C)	41.9 %

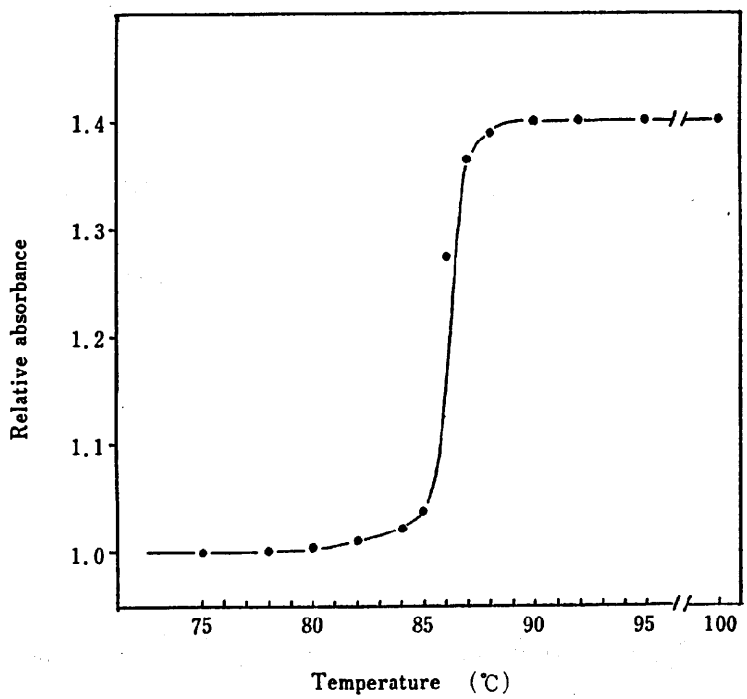

Fig. 7. Thermal denaturation of phage NP-1 DNA.

Amino acid composition of phage protein

As shown in Fig. 8, phage NP-1 cl was purified with high recovery by column chromatography. Table 6 shows the results of amino acid analysis of the purified phage. The amino acid composition of the phage was similar to those of *coli* phages,[7,21] expect that aspartic acid, glutamic acid, glycine and threonine were somewhat at a high level and arginine, histidine, lysine and methionine at a slightly low level.

Table 6. Amino acid composition of phage NP-1 cl.

Amino acids	NP-1 cl* (%)	Difference to average T_3 and T_4 (%)†
Alanine	10.1	+0.45
Arginine	4.0	−2.75
Aspartic acid	15.0	+3.36
Glutamic acid	13.4	+2.15
Glycine	10.0	+1.45
Histidine	0.6	−1.55
Leucine	7.6	−0.55
Isoleucine	3.2	−1.10
Lysine	4.9	−2.50
Methionine	0.2	−1.20
Phenylalanine	4.4	+0.70
Proline	4.9	+0.30
Serine	4.7	+0.80
Threonine	7.7	+1.55
Tyrosine	4.5	+0.55
Valine	5.8	−0.65
Cystine	—	—
Tryptophan	—	—

* The values are expressed as per cent of recovered amino acids.
† NP-1 cl (%)-average *coli* phage T_3 and T_4 (%).[7,21]

Lysogeny

Phage NP-1 formed a turbid plaque and UV-inactivated phage had no killing action on host bacteria (Table 8), The results indicate that the phage is lysogenic. Table 7 shows the frequency of lysogenization of phage NP-1 and NP-1 cl. The host bacteria were lysogenized with phage NP-1 at a frequency of about 3.5×10^{-1} through various multiplicities of infection. NP-1 cl could also lysogenze at a low frequency of 5×10^{-3}. As seen in Fig. 9, the lysogenic bacteria produced usually

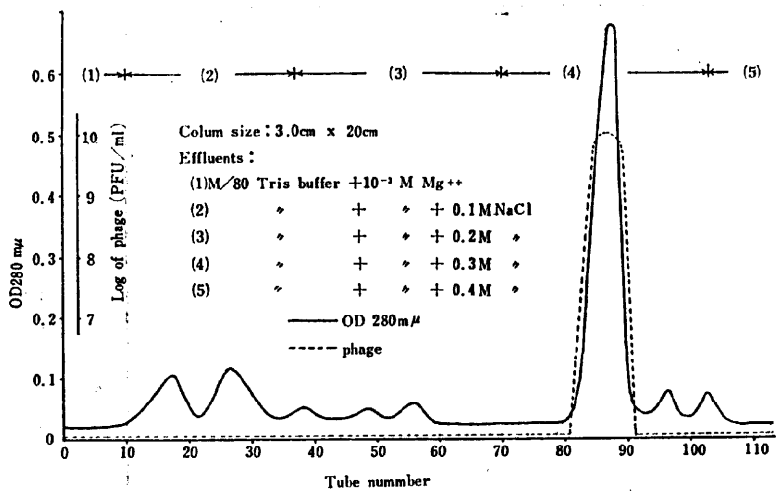

Fig. 8. Purification of phage NP-1cl by DEAE-cellulose column chromatography.

Table 7. Lysogenization of the bacteria with NP-1 and NP-1cl.

Phage	Moi*	Frequency of lysogenization
	100	1.5×10^{-1}
NP-1	10	3.5×10^{-1}
	3	3.5×10^{-1}
NP-1cl	100	5.0×10^{-3}

* Multiplicity of infection.

The frequency of lysogenization is represented as the number of lysogenic cells divided by the number of cells present at the time of infection.

Table 8. The determination of killing action of UV-inactivated phage on host bacteria.

Inactivation of phage by UV irradiation			Number of host bacteria		
UV dose (sec)	Phage (PFU/ml)	Survivals (%)	Before infection (cells/ml)	After infection (cells/ml)	Killing rate (%)
0	4.0×10^{10}	100	2.2×10^{8}	2.3×10^{7}	89.6
50	3.5×10^{9}	8.8	2.2×10^{8}	6.9×10^{7}	58.7
100	1.8×10^{8}	0.5	2.2×10^{8}	2.0×10^{8}	9.0
150	1.0×10^{6}	0.003	2.2×10^{8}	2.1×10^{8}	4.5

Phage NP-1 suspension (4×10^{10} PFU/ml) was irradiated by UV for the indicated times. To 1 ml of the host culture (2.2×10^{8} cells/ml) was added 0.1 ml of the UV-irradiated phage suspension; a ratio of phage particles/bacterium was about 18. The mixture was allowed to stand at 37°C for 10 min and plated after appropriate dilution, and the plates were incubated overnight to determine the survived cells.

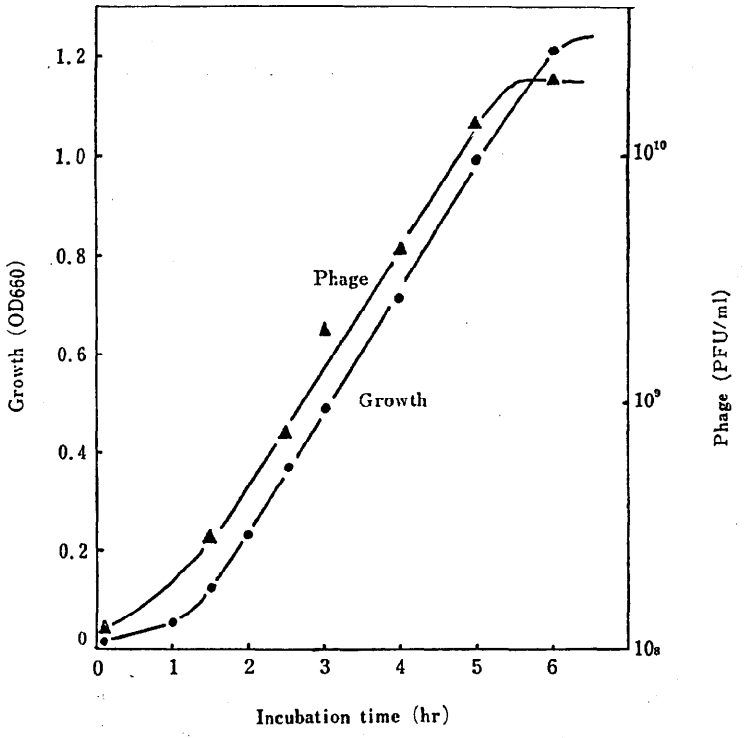

Fig. 9. Growth and phage production of a lysogenic strain.

high titers of about 10^{10} PFU/ml after 5-hr incubation, suggesting that they were spontaneously lysed at a high frequency. The prolonged incubation of the lysogenic bacteria on slant-agar medium tended to decrease considerably in the phage production. However, when the lysogenic bacteria were successively transferred on new slant-agar medium within a week, the phage producibility was retained at a maximum level. In addition, the lysogenic bacteria at the logarithmic phase of growth were subject to anaerobic lysis. For instance, the 4-hr culture grown in PY medium lysed within 1 hr even by stopping to shake.

Takahashi[28] and Bott et al.[4] have found that temperate phages PBS1 and SP 10 can not perform a true lysogeny, since the spores which were derived from these lysogenic bacteria composed of each 50 % of lysogenic and nonlysogenic one and the incubation of these lysogenic bacteria in the presence of antiphage serum decreased easily in the number of lysogenic cell. Spores from the bacteria lysogenized with NP-1 could contain only a few per cent of lysogenic spores. These results

suggest that this phage can not perform a true lysogeny such as λ phage[15] and may belong to pseudolysogenic group as well as phage PBS 1 and SP 10. Unusual properties of this lysogenic bacteria will be investigated and described elsewhere.

Failure of transduction with phage NP-1

The transduction with NP-1 was studied by the same manner as described by Thorne,[32] expect that minimum medium was supplimented with biotin, which was esential for the growth of strain No. 3. A few differnnt media were also used. As marker strains were used some leucine and arginine less mutants and several unknown mutants which were obtained by treatment with N-methyl-N-nitroso guanidine.[2] So far examined, the transduction have not yet succeeded.

Summary

A bacteriophage of *Bacillus natto* was isolated and investigated on the following properties: host range, stability, adsorption kinetics, burst size, thermal inactivation, UV inactivation, DNA base composition, amino acid composition of whole phage, lysogeny and some other characteristics.

This phage was temperate and often produced a clear plaque mutant. The phage attacked against *B. natto*. The lysogenization frequency was 3.5×10^{-1} and the burst size was about 50. The phage was stable at the pH range between 5.0 to 9.0, inactivated at 50°C and was very resistant to UV. The phage DNA did not contained an unusual base, and its guanine plus cytosine contents were about 41 %. Amino acid composition of whole phage protein was nearly similar to those as described with regard to T series of *coli* phage. Attempt to transduce a few amino acid markers with phage was unsuccessfully made.

Acknowledgment

The authers are indebted to Prof. H. Fujii, Fukuoka Women's University for a gift of *Bacillus natto* strains and his helpful discussion, and thank Prof. Y. Ikeda, Tokyo University and Prof. K. Watanabe, Fukuoka University for supplying many other *Bacillus* strains. Thanks are also expressed to Prof. H. Endo for giving *Escherichia coli* strain B and *coli* phages, and Mr. K. Kurachi, for operating amino acid autoanalyzer.

References

1) Adams, M. H., "Bacteriophage" Interscience Publishers, Inc., New York, 1959, p. 443.
2) Adelberg, E. A., M. Mandel, and G. C. C. Chen, Biochem. Biophys. Res. Comm., **18**, 788 (1965).
3) Anfinsen, C. B., Fed. Proc., **20**, 634 (1961).
4) Bott, K. and B. Strauss, Virology, **25**, 212 (1965).
5) Brodetsky, A. B. and W. R. Romig, J. Bact., **90**, 1655 (1965).
6) Burton, K., Biochem. J., **62**, 315 (1956).
7) Fraser, D. and E. A. Jerrel, J. Biochem, **25**, 29 (1953).
8) Fukuda, S., Nippon Nogeikagaku Kaishi, **29**, 743 (1955).
9) Fujii, H., K. Oki, M. Makino, G. Akuno, and L. Takeya, *ibid.*, **41**, 39 (1967).
10) Harm. W., Z. Verebungsl., **94**, 67 (1963).
11) Kallen, R. G., M. Simon, and J. Marmur, J. Mol. Biol., **5**, 248 (1962).
12) Kinoshita, I. and T. Okumura, Nippon Nogeikagaku Kaishi, **29**, 322 (1958).
13) Kitahara, K. and T. Kaneko, *ibid.*, **32**, 411 (1958).
14) Koch, G. and W. J. Dryer, Virology, **6**, 291 (1958).
15) Lwoff, A., Bacteriol Rev., **17**, 269 (1953).
16) Mahler, I., Biochem. Biophys. Res. Comm., **21**, 384 (1965).
17) Mandel, J. D. and A. D. Hershey, Anal. Biochem., **1**, 66 (1960).
18) Marmur, J. and P. Doty, J. Mol. Biol., **5**, 109 (1963).
19) Murphy, J. S., Virology, **4**, 563 (1958).
20) Okubo, O. and W. R. Romig, J. Mol. Biol., **14**, 130 (1965).
21) Polsen, A. and R. W. G. Wyckoff, Science, **108**, 501 (1948).
22) Ralston, D. J., B. Bear, M. Lieberman, and A. P. Krueger, J. Gen. Microbiol., **24**, 313 (1961).
23) Romig, W. R. and A. M. Brodetsky, J. Bact., **82**, 135 (1961).
24) Roscoe, D. H. and R. G. Tucker, Biochem. Biophys. Res. Comm., **16**, 146 (1964).
25) Spizizen, I., Proc. Natl. Acad. Sci., **44**, 1072 (1958).
26) Takagi, J., J. Gen. Appl. Microbiol., **8**, 214 (1962).
27) Takahashi, I., Biochem. Biophys. Res. Comm., **5**, 171 (1961).
28) Takahashi, I., J. Gen. Microbiol., **31**, 211 (1963).
29) Takahashi, I. and J. Marmur, Biochem. Biophys. Res. Comm., **10**, 289 (1963).
30) Takahashi, I. and J. Marmur, Nature, **197**, 794 (1963).
31) Thorne, C. B., Fed. Proc., **20**, 254 (1961).
32) Thorne, C. B., J. Bact., **83**, 106 (1962).
33) Volkin, E. and E. W. Cohen, Methods of Biochemcal Analysis, 1958, Vol. 1, p. 287.
34) Watanabe, K. and K. Yamafuji, Enzymologia, **23**, 353 (1961).
35) Wyatt, G. R., Biochem. J., **48**, 584 (1951).