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STUDIES ON VEILLONELLOPHAGES ISOLATED FROM WASHINGS OF HUMAN ORAL CAVITY

BY

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ABSTRACT

Virulent phages specific for *Veillonella* were isolated from washings of the oral cavity of 200 individuals. These phage strains were classified into two major groups, A (N1-11) and B (19-36), by their plaque morphology and serological characters.

By the susceptibility to these phages, 21 strains of genus *Veillonella* were classified into four types (type I to IV). Type I corresponded to *V. parvula*, type II to *V. reniformis*, type III to *V. alcalescens*, and type IV to other strains of *Veillonella*.

The growth curve of veillonellophage N2, N11, and N20 propagated on each of their host *Veillonella* revealed that they had a latent period of 55 min to maximum of 100 min, burst size ranging 40–120 and eclipse period of 20 to 100 min.

Electron microscopy revealed that the group A phage had a hexagonal head of 670 Å diameter and wedge-shaped tail of 180 Å phage length and 90–180 Å width at the base. They were classified as type C according to Bradlay's definition. Group B phages with a hexagonal head of 550 Å diameter, and a striated tail of 1300 Å length and 70 Å width were classified as type B of Bradley's definition.

More than 90% of group A phages and their host *Veillonella* were inactivation by ultraviolet (UV) irradiation, but group B phages and their host were comparatively resistant to UV irradiation. Photoreactivation of veillonellophages were not observed under aerobic and anaerobic condition.

INTRODUCTION

The phage was first identified and disignated by Twort[1] and d'Herelle[2] when they found the bacteriolysis of *Shigella* by an ultramicroscopic parasite. Following their pioneering works, various types of phage have been reported by Boyd[3], Craigie and Yen[4], Tokunaga[5] and Mohony[6]. However their host bacteria were all aerobic bacteria or facultative anaerobic bacteria, and no phage was reported on anaerobic cocci until Shimizu[7] found a temperate phage specific for genus *Veillonella*. His observation that the phage was present for anaerobic cocci such as genus *Veillonella* is considered to be important in oral bacteriology, since genus *Veillonella* is predominant in oral cavity.

In a previous study, Hiroki and Totsuka[8], reported the isolation of a virulent phage from washings of oral cavity, specific for the genus *Veillonella*. It was shown that they were not a temperate phage, but virulent phage, since no phages were isolated from culture filtrates of the bacterial strains used. This phage strain was classified into two major groups by their plaque morphology, host range, and serological characteristics.

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This paper discribes detailed nature of these phages, including their phage typing, their mode of multiplication in the host cell, their morphology by electron microscopy, and the effect of ultraviolet (UV) irradiation and photoreactivation, in order to elucidate the relationship to their host cells.

Materials and Methods

Bacterial strains and phages

Strains of *Veillonella* used as the host bacteria were isolated from human oral cavity; strains number 35-16, 38-7, 43-9, 44-14, 51-24, 76-11, 85-3, 93-19, 96-12, 107-14, 109-9, 130-11, 149-5, and 150-5. Standard strains were obtained from American Type Culture Collection, *Veillonella parvula* ATCC 10790, 17743, and 17744, and *Veillonella alcalescens* ATCC 17745, 17746, 17747, and 17748. A total of 21 strains were used.

Twenty-five strains of veillonellophages were isolated from oral washings of 200 students (age range 18–26 years) as reported previously, which were designated as N1, N2, N3, N4, N6, N10, N11, N19, N20, N21, N22, N23, N24, N25, N26, N27, N28, N29, N30, N31, N32, N33, N34, N35, and N36.

For the control strains of UV irradiation experiment, strains of *Mycobacterium smegmatis* ATCC 607, *Escherichia coli* B, mycobactreiophages ~~AD and~~ D29, and coliphage T2 were used.

Veillonella strains in Rogosa's broth (10 ml) was added to the washings (100 ml for a single experiment) and incubated anaerobically at 37°C. Incubated media were filtrated through a Millipore filter (0.22 μm porosity). The filtrate was spotted on a double agar layer plate containing *Veillonella* of the same strain used for incubation. The area of the plate where the bacteriolysis occurred was collected, suspended in Rogosa's broth, and filtrated. The filtrate was incubated again on the same double agar layer plate. These filtrations were repeated until a single plaque was obtained.

Media

The medium used was Rogosa's liquid media[9] containing 5 g of trypticase (Difco), 3 g of yeast extract (Difco), 0.75 g of sodium thioglycollate (Difco) and 12.5 ml of sodium lactate (50%, Wako), in 1000 ml of distilled water (final pH 7.2). All the broth used was boiled and immediately cooled before inoculation in order to reduce the level of dissolved oxygen. Fifteen grams of Bactoagar (Difco) for solid media or 6 g Bactoagar for semisolid media was added to 1000 ml Rogosa's liquid media.

Incubation technique

All the incubations in this study were made at 37°C for 2 or 3 days in an anaerobic incubator (Perfect Chamber, Hirasawa, Tokyo). The chamber was evacuated to the level of 10 mm Hg and then filled with 83% nitrogen, 7% hydrogen, and 10% carbon dioxide gas until the atmospheric pressure was obtained. Platinum cold catalyst (BBL) was used as the catalyst in the incubator to remove the remaining oxygen. Gas Pak system (BBL) and steel wool method were used for a small number of plates and tubes.

Propagation of the phage

About 1 ml of the suspension of plaque prepared as already stated was plated with indicator strains by the double-agar layer method. The resulting plate thus prepared which usually contained many plaques was extracted with a few milliliters of the broth for several hours. After removal of bacteria by centrifugation at 3000×g for 15

min, the supernatant was filtered through a Millipore filter (0.22 μm porosity) and used again to lyse a larger number of plates.

Titration of phage

About 3 ml of melted semisolid medium was cooled to 50°C, and inoculated with 0.3 ml of a concentrated suspension of the indicator bacteria, 0.1 ml of phage suspension was added, and the entire mixture was poured over the surface of a hardened layer of a solid medium. After the upper agar layer had solidified the plates were incubated and assayed for the plaque-forming activity.

Phage typing

Approximately 3 ml of melted semisolid medium was cooled to 50°C and inoculated with 0.3 ml of a concentrated suspension of the strains for typing. The entire mixture was poured over the surface of a hardened layer of solid medium and it was dried at 37°C for 1 hr before use.

For determination of the routine test dilution (RTD), the stock phage suspension was diluted serially at a multitude of ten with Rogosa's broth, and each diluted suspension was spotted on the plate. The highest dilution that gave confluent lysis was adopted as 1 RTD suspension. One drop of 1 RTD suspension (approximately 0.01 ml each) of phages was then applied to each separate area on the plate containing *Veillonella*. The drops were allowed to dry and the plates were then incubated anaerobically at 37°C for 2 days.

Identification of Veillonella used for phage typing

Gas production, gelatin liquefaction, indole production, nitrate reduction, H_2S production, and catalase activity were examined by the method of Rogosa[9].

One-step growth exermination

A method similar to that described by Ellis and Delbrück[23] was employed. Phage suspension was added to an exponentially growing culture of the indicator strain (5–10×10^8 cells/ml) at a multiplicity of infection (MOI) of about 0.1. After 30 min of adsorption in an anaerobic condition, phage anti-serum was added to the adsorption mixture to inactivate unadsorbed phages. After 10 min, the mixture was diluted 10^4 times and 2 ml was distributed into each of the test tubes. At various time intervals (30–60 min), a sample tube was withdrawn, divided into two portions, and one drop of chloroform was added to one of them. The number of infective centers in both portions was measured.

Electron microscopy

Fresh suspension of veillonellophage (10^{10}/ml) was prepared from neutral 0.1 M ammonium acetate solution as follows: The phages were sedimented by centrifugation at about 30,000×g for 2 hr at 4°C. The sediment was resuspended in ammonium acetate solution and two further cycles of ultracentrifugation and resuspension produced a clean enough preparation for electron microscopy. Phage suspension in 0.1 M ammonium acetate solution (final concentration 10^{11}–10^{12}/ml) was stained negatively with neutral 2% potassium phosphtungstate solution (PTA) according to the method of Brennes and Horne[10].

Ultraviolet (UV) irradiation and photoreactivation

The source of UV was a germicidal lamp (National, GL 15W Matsushita Electric

Co., Osaka), emitting the rays predominantly at 253.7 mn and having an intensity of 30 erg/mm²/sec at a distance of 50 cm. Routinely, suspension of a phage (10^8/ml) in buffered physiological saline (pH 7.2) in an open petri dish was placed on an oscillating platform at a distance of 50 cm from the UV source and stirred gently during irradiation. After 5 min, 0.1- to 1-ml samples were withdrawn and assayed for the plaque-forming activity. Similarly, the indicator strains in late logarithmic growth (10^8/ml) were irradiated with UV ray.

For detection of photoreactivation, radiant energy was supplied by a lamp containing two 15W cool white fluorescent tubes (National Highlight White FL 15W). Duplicate sets of samples for each irradiation were illuminated at a distance of 20 cm from the light source. The illumination for 2 hr at 37°C was started immediately after plating. The dark control samples were processed in the same way as the illuminated samples, except that the plates were wrapped in opaque aluminium foil. The number of plaques in three plates in both illuminated and dark control were counted. The photoreactivation procedure was carried in both aerobic and anaerobic environment. Percentage survival (ratio of difference between the

Table 1. Phage typing of genus *Veillonella*

Bacteria \ N phages	1	2	3	4	6	10	11	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
10790	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
17743	##	##	##	##	##	##	##	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
17744	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
17745	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
17746	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
17747	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
17748	##	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
35-16	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
38-7	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
43-9	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
44-14	−	−	−	−	−	−	−	##	##	##	##	+	###	###	###	###	##	##	+	##	###	##	##	###	###
51-24	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
76-11	−	−	−	−	−	−	−	###	###	###	###	###	###	###	###	###	###	###	###	###	###	###	###	###	###
85-3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
93-19	##	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
96-12	−	−	−	−	−	−	−	###	###	###	###	###	###	###	###	###	##	##	+	###	###	###	###	###	###
107-14	+	##	###	###	+	##	###	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
109-9	−	−	−	−	−	−	−	###	##	##	###	##	###	###	##	##	###	###	###	##	###	###	###	###	###
130-11	###	###	###	###	###	###	###	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
149-5	−	−	−	−	−	−	−	###	###	###	###	###	###	##	###	##	###	###	+	###	###	###	###	###	###
150-5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
IRTD (/ml)	2 × 10^6	4 × 10^4	1 × 10^5	2 × 10^8	3 × 10^8	2 × 10^6	2 × 10^6	3 × 10^4	1 × 10^5	3 × 10^3	7 × 10^4	5 × 10^5	7 × 10^5	2 × 10^3	3 × 10^4	2 × 10^4	8 × 10^3	1 × 10^3	2 × 10^2	8 × 10^4	1 × 10^5	1 × 10^5	4 × 10^4	2 × 10^5	2 × 10^5

Notation of recording lysis is as follows:
Strog lysis (complete lysis in the area dropped)
Moderate lysis (20–50 plaques)
+ Weak lysis (less than 20 plaques)
− No lysis (no plaques)

Table 2. Relationship between the phage typing and classification made from biochemical activities

Types	Strains	Gas production	Gelatin liquefaction	Indole production	Nitrate reduction	H_2S production	Catalase test	
	17743	+	−	−	−	+	−	*V. parvula*
I*	107-14	+	−	−	−	+	−	*V. parvula*
	130-11	+	−	−	+	+	−	*V. parvula*
	44-14	−	−	+	+	−	+	*V. reniformis*
	76-11	−	−	+	+	−	+	*V. reniformis*
II**	96-12	−	−	+	+	−	+	*V. reniformis*
	109-9	−	−	+	+	−	+	*V. reniformis*
	149-5	−	−	+	+	−	+	*V. reniformis*
	38-7	+	−	−	+	+	+	*V. alcalescens*
III***	93-19	+	−	−	+	+	+	*V. alcalescens*
	17748	+	−	−	−	+	+	*V. alcalescens*
	35-16	−	−	−	−	+	+	*V. orbiculus*
	43-9	+	−	−	−	+	+	*V. alcalescens*
	51-24	+	−	−	−	+	+	*V. alcalescens*
IV****	85-3	+	−	−	−	+	+	*V. alcalescens*
	150-5	+	−	−	−	+	+	*V. alcalescens*
	10790	+	−	−	−	+	−	*V. parvula*
	17744	+	−	−	+	+	−	*V. parvula*
	17745	+	−	−	+	+	+	*V. alcalescens*
	17746	+	−	−	+	+	+	*V. alcalescens*
	17747	+	−	−	−	+	+	*V. alcalescens*

* Group A phages, sensitive
** Group B phages, sensitive
*** Group A phages, a part sensitive
**** Group A. B phages, no sensitive

illuminated and the dark control to the dark control expressed in percent, (Np-Nd) $\times 100/Nd$) is shown in Fig. 7.

Results

Phage typing of genus Veillonella

As shown in Table 1, six strains of *Veillonella* were lysed by group A phages, three of which strongly and other three weakly. No lysis of ATCC strains other than the indicator strain (17743) was observed by the phage N1, N2, N3, N4, N6, N10, and N11, except that only ATCC 17748 strain showed a weak lysis by the group of phages. Another group of phages (group B) did not lyse any of the ATCC strain, but did five strains of newly isolated *Veillonella* 44-11, 76-11, 96-12, 109-9, and 149-5. No other strains of the bacteria used were lysed by both groups of phage.

These finding lead us to classify 21 strains of genus *Veillonella* into four types, and the result of typing of *Veillonella* by their susceptibility to the phage in shown in Table 2. Type I of *Veillonella* was related to *V. parvula*, type II to *V. reniformis*, and type III to *V. alcalescens*. They were considered to be a subtype of I. However, some strains of the species belonging to *Veillonella* were not lysed by both types of phage and were classified as type IV.

One-step growth curve of veillonellophage N2 phage (group A):

One-step growth

experiment was carried with typical strains of the phage. One-tenth ml of N2 phage (1.5×10^9/ml) suspension was mixed with 0.9 ml of the indicator strain (ATCC 17743, 2.1×10^9/ml viable cells). MOI was approximately 0.08, the latent period was 55 min, and the burst size was 70. It is shown in Fig. 1 that a premature lysis can be induced within 20 min when chloroform was added to the medium. The indicated that eclipse period of N2 phage was 20 min.

N11 phage (group A): One-tenth ml of N11 phage (1.5×10^9/ml) suspension was mixed with 0.9 ml of the indicator strain (ATCC 17743, 2.4×10^9/ml viable cells), and MOI was estimated approximately as 0.06. The latent period was longer than N2 phage, 80 min, and the rise period was about 45 min, burst size was 120, and the eclipse period was 50 min as shown in Fig. 2.

N20 phage (group B): One-tenth ml of N20 phage (1×10^7/ml) suspension was mixed with 0.9 ml of the indicator strain (76-11, 1.3×10^7/ml viable cells), and MOI was approximately 0.08. This had the longest latent period among the three strains of phage examined, 100 min, and the burst size was 40. Premature lysis with chloroform was also noted, giving an eclipse period of about 70 min as shown in Fig. 3.

One-step growth experiments were also attempted under aerobic condition, but, no phage multiplication was observed with any of the three strains of phage.

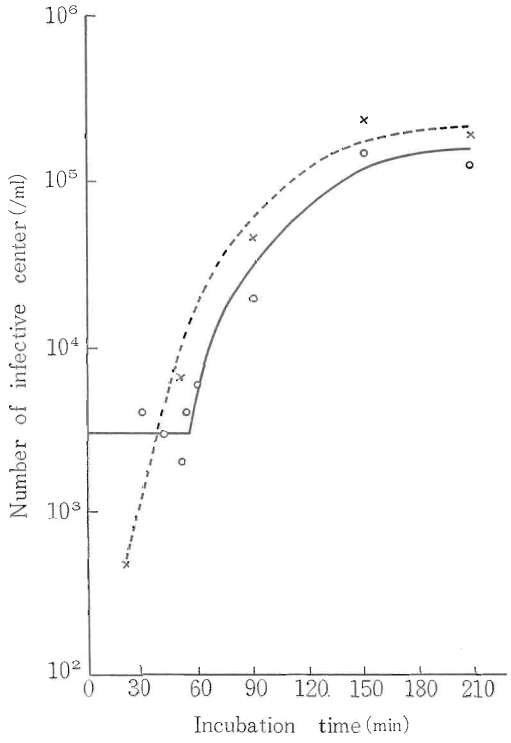

Fig. 1. One-step growth curve of veillonellophage
Phage N2
○——○ Number of infective centers without chloroform treatment
×---× Number of infective center with chloroform treatment

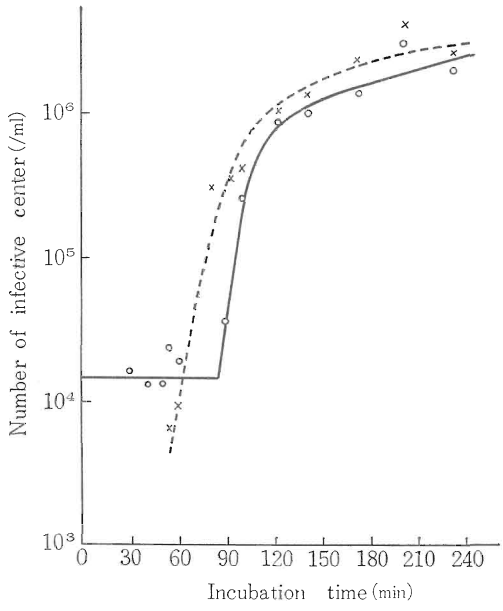

Fig. 2. One-step growth curve of veillonellophage
Phage N11
○——○ Number of infective centers without chloroform treatment
×---× Number of infective center with chloroform treatment

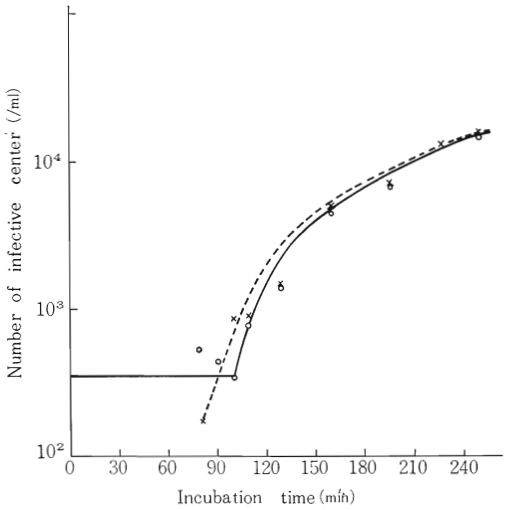

Fig. 3. One-step growth curve of veillonellophage
Phage N20
○——○ Number of infective centers without chloroform treatment
×---× Number of infective center with chloroform treatment

Electron microscopy

Electron micrographs of typical strains of the phages in group A (N2, N11) and group B (N20, N24) are shown in Fig. 4. N2 phage (group A) had a head and a short tail. The head measured 670 Å in diameter and showed hexagonal appearance. The tail showed the so-called wedge shape and was 180 Å in length and 90–180 Å in width at the base (Fig. 4 a,b). The structure of N11 phage (group A) was very similar to that of N2 phage, but a base plate-like structure was often observed in the former group (Fig. 4 c,d). N20 phage (group B) had a regular shaped head with a diameter ranging from 560 to 600 Å. The tail of N20 phage was about 1300 Å long and 70 Å wide, and possessed about 30 striations. Base plate of 250 Å width and a spike-like structure were also observed (Fig. 4 e,f). N24 phage (group B) showed a structure similar to that of N20. This phage had a head of 700 Å in length and 620 Å in width, in a hexagonal shape, and had a tail of 1200 Å in length and 90 Å in width (Fig. 4 g,h).

Ultraviolet irradiation

Effect of UV irradiation of indicator bacteria: Percentage survival after UV. irradiation of *Mycobacterium smegmatis* ATCC607, *Escherichia coli* B, *Veillonella parvula* ATCC 17743, and *Veillonella reniformis* 76-11 is shown in Fig. 5. The survival curve for *M. smegmatis* showed a distinct shoulder in the low-dose region, meaningly, the organism was been less effective for short-time irradiation. The survival curves for *E. coli* B and *M. smegmatis* agree relatively well with the result pub-

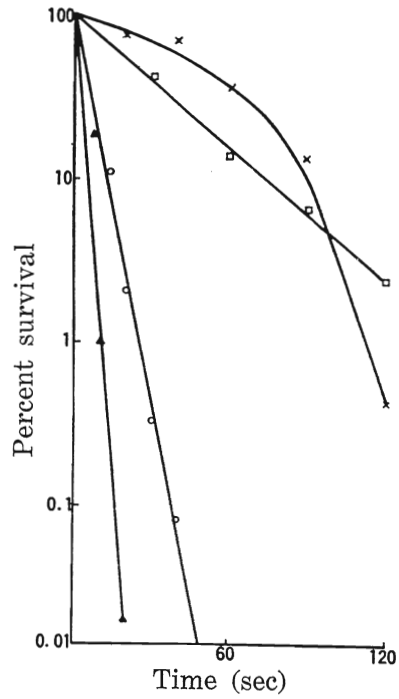

Fig. 5. Eflect of UV iradiation on indicator bacteria
▲——▲ E. coli B
×——× M. smegmatis ATCC607
○——○ V. parvula ATCC17743
□——□ V. reniformis 76-11

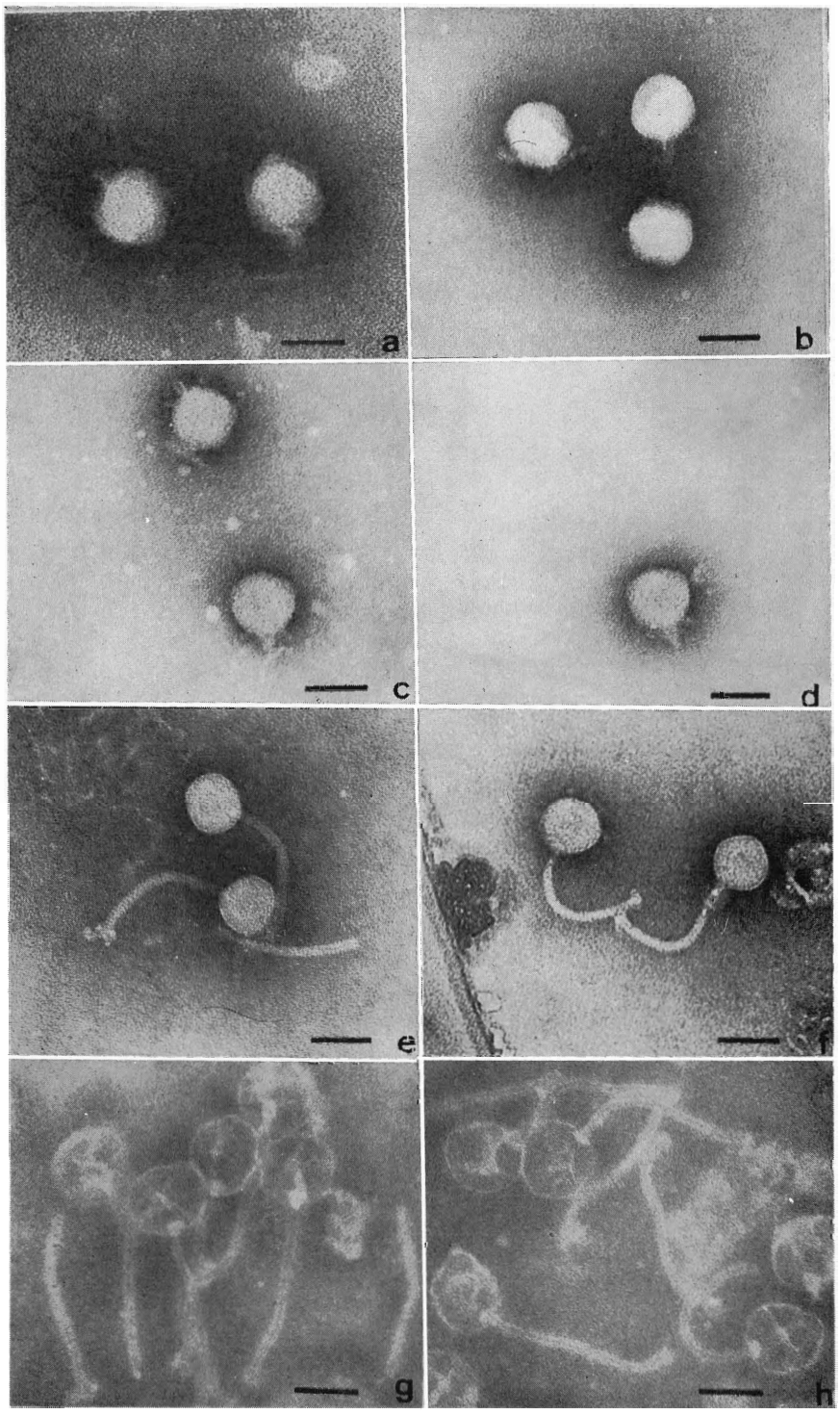

Fig. 4. Electron micrograph of veillonellophage negatively stained with PTA scale 500Å.
a), b). N2 c), d). N11 e), f). N20 g), h). N24

lished by Sellers *et al.*[11]. Time required for 99% inactivation of each bacteria by UV irradiation were 10 sec in the case of *E. coli* B and 110 sec for *M. smegmatis*. Remarkable difference in susceptibility to UV irradiation was observed between two strains of *Veillonella* used. Strain ATCC 17743 showed a survival curve nearly the same as that of *E. coli* B, indicating distinctly higher susceptibility of this strain than the strain 76-11 and *M. smegmatis*.

Effect of UV irradiation on phages: Survivable curves of phage T2 and D29 which were used for control experiment agreed relatively well with the data published by Sellers *et al.*[11]. The time required for 99% inactivation of T2 and D29 were 7 sec and about 160 sec, respectively.

In the case of veillonellophage, two different kinds of survival curve were obtained. Phage N2 and N11 showed a linear declining curve, and N20 and N24 showed curve having two inflection points. The survival curves for N2 and N11 resembled that of T2, and the latter two resembled D29. This result indicated that the susceptibility under UV irradiation is correlated to serological groups, since phage N2 and N11 belong serologically to group A, and N20 and N24 to B group.

Photoreactivation: Photoreactivation of veillonellophages was not detected in this experimental condition (Fig. 7). The experiments were carried in aerobic and anaerobic conditions, both giving the same negative results as shown in Table 3. Con-

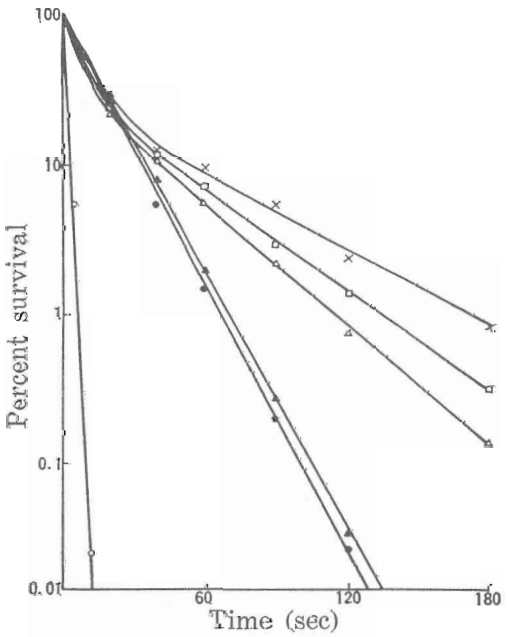

Fig. 6. Effect of UV irradiation on phages

×——×	Mycobacteriophage	D29
□——□	Veillonellophage	N20
△——△	,,	N24
▲——▲	,,	N11
●——●	,,	N 2
○——○	Coliphage	T 2

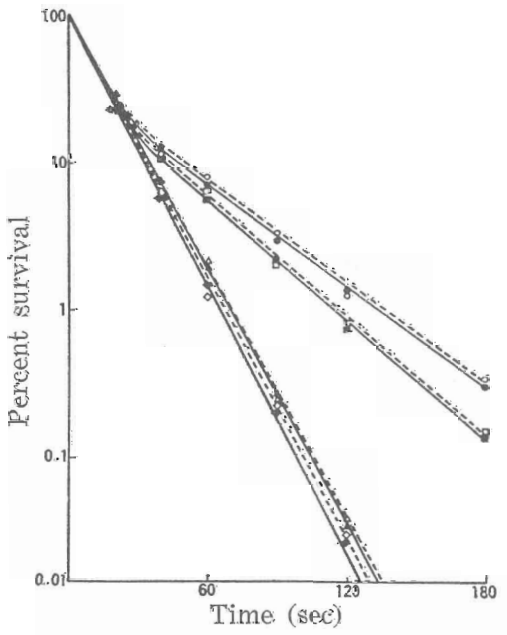

Fig. 7. Photoreactivation of veillonellophage after UV irradiation.

●——●	N20 control	(Np-Nd)×100/Nd
○——○	,, illumination	(%)
■——■	N24 control	Np: A number of
□——□	,, illumination	plaques in the il-
▲——▲	N11 control	luminated.
□——□	,, illumination	Nd: A number of
◆——◆	N 2 control	plaques in the
◇——◇	,, illumination	dark.

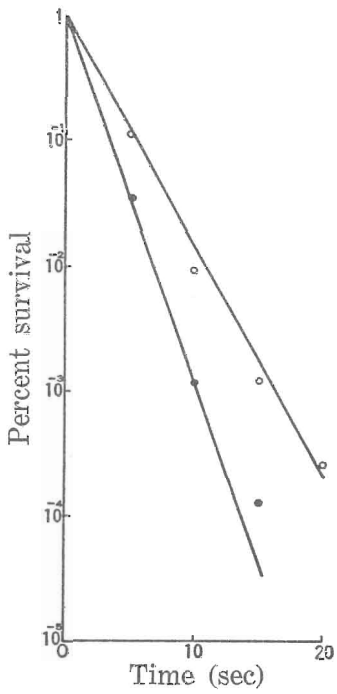

Fig. 8. Photoreactivation of T2 phage after UV irradiation
●——● Dark control
○——○ Illuminated after UV irradiation

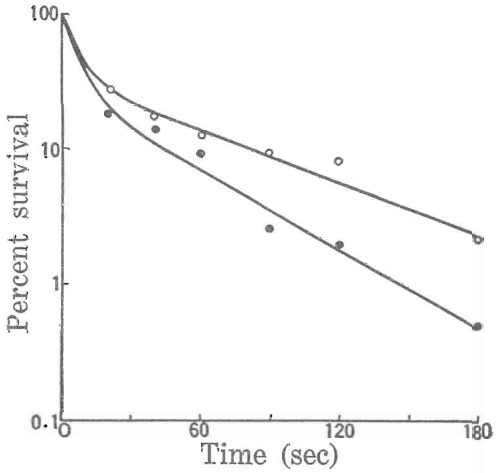

Fig. 9. Photoreactivation of D29 phage after UV irradiation
●——● Dark control
○——○ Illumination after UV irradiation

trol T2 and D29 phages recovered the activity by illumination of light (Figs. 8 and 9), as reported by Tokunaga and Seller[22].

Table 3. Photoreactivation under aerobic and anaerobic condition of UV irradiated phages

Phages	Survival of phages (%)		
	Dark controls	Illumination	
		Aerobic	Anaerobic
T2	0.16	0.86	0.87
D29	2.2	10.8	10.2
N2	0.018	0.017	0.016
N20	1.6	1.53	1.54

UV irradiation time was 10 sec for T2 phage and 120 sec for others

Discussion

Phage typing of genus Veillonella

Genus *Veillonella* was first designated for a group of anaerobic cocci characterize by non-motility and negative gram staining. Pelcza[12] classified them into six species by gas production, gelatin liquefaction, and indole production of *V. parvula*, *V. alcalescens*, *V. discoides*, *V. reniformis*, *V. orbiculus*, and *V. vulvovaginitidis*.

In the present work, genus *Veillonella* was classified into four groups (type I to IV) by their susceptibility to veillonellophage A and B, as described in the Result section. The relationship among these four types and the classification of *Veillonella* proposed by Pelcza are as follows: Type I to *V. parvula*, type II to *V. reniformis*, type III to *V. alcalescens*, and type IV includes *V. parvula*, *V. alcalescens*, and *V. orbiculus*. The significance of this new classification of *Veillonella* by their susceptibility to phages can be emphasized in that they are based on the fundamental genetic mechanism of each *Veillonella* species.

There has been some confusion in the classification of *Veillonella*, since Rogosa[13]

reported recently that *Veillonella* can be classified into only two species by their lipid production and excluding the four species described by Pelcza from genus *Veillonella*. The new classification described in the present paper will give some clue to the understand of species specificity of genus *Veillonella*, suggesting the presence of at least four kinds of species in this genus.

One-step growth curve

No information has been available on the cycle of infection and multiplication of phage to anaerobic bacteria except the work by Mahony and Kals which showed the growth curve of a temperate phage of *Clostridium perfringens*[14].

The present work clearly showed that one-step growth curve of veillonellophages was almost the same as that of the phage for aerobic bacteria with a whole adsorption period of 30 min and no rise period. The burst size of phage N11 was almost the same as that of T4 phage. From these results, it can be concluded that the biosynthesis of phage particles within the infected cell of *Veillonella* under the anaerobic condition is essentially the same as in the case of aerobic bacteria.

Electron microscopic structure of veillonellophage

The difference in structure of veillonellophages agrees with their serological classification. The group A phage has a hexagonal head of 670 Å in diameter and a wedge-shaped tail, while the group B phage has a hexagonal head, with a diameter ranging from 560 to 600 Å, and a longer striated tail of 1300 Å in length and 70 Å in width. The group A phages we found showed the same morphology as that of *E. coli* phage T3 and T7, *B. subtilis* phages ϕ29 and Nf[19] and *Salmonella* phage P22[17], all having a short noncontractile tail. They can be morphologically classified as type C, accordingly to the classification of Bradley[15]. Morphology of the group B phage was the same as that of *E. coli* phage T1 and T5, having a long noncontractile tail, and can be classified morphologically as type B of Bradley's definition.

According to Ackerman, the morphological group C phages were divided into type C1, C2, and C3 by the structure of their tail. The group A phages we found belong to type C1 of Ackerman's definition. The type C1 phages have been recorded in some bacteria. *Pseudomonas* phage gh-1[16] has a hexagonal head of 500 Å in diameter, and a wedge-shaped tail. *Salmonella* phage P22[17] has hexagonal head of 600 Å in size. They have a tail assembly showing 3 or 6 pins in profile or in end-on view. The spike-like structure observed in group A veillonellophages in this study might correspond to these pin structures.

Thus, type B veillonellophages can be classified as B1 according to the definition of Bradley and Ackerman[15]. The same B1 phage group includes *E. coli* phage T1 and T5, *Salmonella* phage, *Streptococcus faecalis* phage, and many others[18]. From these results, it can be said that the structure of veillonellophage A and B is a rather common one which can be observed in large number of bacteriophages.

Effect of ultraviolet on veillonellophage

Effect of UV irradiation on phage was investigated to characterize the veillonellophages. It was clearly shown that veillonellophage N2 and N11 in group A had a susceptibility to UV irradiation different from veillonellophage N20 and N24 in group B. This difference of susceptibility

is probably related to the difference in DNA structure of the phages, since it is widely accepted that the base composition of DNA of a phage is directly related to their susceptibility to UV and a high content of thymine is known to correspond to high susceptibility[20-22].

It is interesting to note that *Veillonella* which can be lysed by veillonellophages with high susceptibility to UV irradiation also has a high susceptibility to UV irradiation, as shown in Figs. 5 and 6. This coincidence in susceptibility between the phages and their indicator *Veillonella* suggests that characteristics of DNA composition in the phage might reflect that of indicator bacteria, but further work of biochemical nature will be required to clarify the detailed relationship between veillonellophage and their host genus *Veillonella*.

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