A Giant Phage Taillike Particle of Clostridium Saccharoperbutylacetonicum ATCC 13564

Ogata, Seiya
Microbial Genetics Laboratory, Institute of Genetic Resources, Faculty of Agriculture, Kyushu University

Nishiyama, Takashi
Microbial Genetics Laboratory, Institute of Genetic Resources, Faculty of Agriculture, Kyushu University

Doi, Katsumi
Microbial Genetics Laboratory, Institute of Genetic Resources, Faculty of Agriculture, Kyushu University

Yoshino, Sadazo
Laboratory of Applied Microbiology, Faculty of Agriculture, Kyushu University

他

https://doi.org/10.5109/24315

出版情報：九州大学大学院農学研究院紀要. 44 (1/2), pp.127-135, 1999-11. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **44** (1·2), 127–135 (1999)

A Giant Phage Taillike Particle of *Clostridium Saccharoperbutylacetonicum* ATCC13564

Seiya Ogata,[1] Takashi Nishiyama,[1] Katsumi Doi,[1] Sadazo Yoshino,[2] Fumio Kato[3] and Hans–W. Ackermann[4]

Microbial Genetics Laboratory, Institute of Genetic Resources,[1] Laboratory of Applied Microbiology,[2] Faculty of Agriculture, Kyushu University, Higashi–ku, Fukuoka 812–8581, Department of Applied Biological Science,[3] Saga University, Saga 840–8502, Japan, and Department of Microbiology,[4] Faculty of Medicine, University of Laval, Quebec, Canada G1k7P4

(*Received July 30, 1999 and accepted August 24, 1999*)

A unique phage taillike particle from *Clostridium saccharoperbutylacetonicum* ATCC13564 has been studied by electron microscopy using negative staining. It was observed in the lysate of phage HM 3 and in the mitomycin C–induced lysate with an inducible phage taillike bacteriocin, clostocin O. It was striated rod 28 nm in diameter and 450 nm in length, ending in three helical or wavy fibers (230×12 nm) which ended in special structures like suckers or funnels. Its contracted sheath was estimated to be 350 nm in length and 32 nm in width.

INTRODUCTION

The bacteriophages (phages) are the viruses infectious to the prokaryotes. Phage research has been, and will continue to be, an important field of microbiology. It has made enormous contributions to molecular biology, biochemistry and genetics, and it is having impact in such diverse field as epidemiology of infectious diseases, agriculture and the fermentation industry (Ackermann and DuBow, 1987a; Ogata, 1982).

The electron microscopy is suitable for the detection of phages and in particular for studying their morphology and physiology. Electron microscopic investigation on the phage lysate and mitomycin C– or UV–induced lysate frequently shows that the existence (or coexistence) of a great variety of phagelike particles, such as heads, long or short tails, and their components or parts (Ackermann and DuBow, 1987a; Bradley, 1967; Tikhonenko, 1970). During the examination on the induction of phage taillike bacteriocin, clostocin O of *Clostridium saccharoperbutylacetonicum* ATCC 13564 by mitomycin C (Ogata *et al.*, 1972; 1978), this strain was found to produce another type of phage taillike particles. The production of the giant tail particles was also induced by phage HM 3 infection. The fine structure of this kind of particles have not been reported in *Clostridium* strains.

This paper deals with the detection and morphological property of the unique phage tail particles. The particles had been found twenty years ago by Ogata and Kato, the authors of these paper, and were recently found individually by Ackermann, a coauthor. Many references for the phagelike particles of various species of *Clostridium* are cited in the reports of Ackermann and DuBow (1987c), Nieves *et al.* (1981) and Ogata and Hongo (1979).

MATERIALS AND METHODS

Bacterium and phages

The strain used was *Clostridium saccharoperbutylacetonicum* ATCC 13564 (strain N1-4) previously found to produce an inducible phage taillike bacteriocin, clostocin O (Ogata *et al.*, 1972). Phage HM 3 (ATCC 13564-B2) was grown on the strain N1-4 (Ogata *et al.*, 1969).

Medium and cultural condition

Growth of the bacterial organisms was made at 30°C under a reduced atmospheric pressure (5 to 10 mm Hg) in TYA medium (Ogata and Hongo, 1973) which contained (g/l distilled water): glucose, 40; Bacto-tryptone (Difco), 6; yeast extract (Daigo Eiyo Kagaku Ltd.), 2; ammonium acetate, 3: KH_2PO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.4; $FeSO_4 \cdot 7H_2O$, 0.01; at pH 6.5. To obtain a young exponential growing culture, fresh medium was inoculated with sufficient organisms to produce an initial optical density (OD) of 0.1 at 660 nm and the culture was incubated until its OD_{660} became 0.25 to 0.3. The OD was measured with a photoelectric colorimeter (Model 7A, Tokyo Koden Ltd.)

Preparation of phage lysate

The lysate of phage HM 3 was prepared by the procedures as described (Ogata *et al.*, 1969).

Induction of clostocin O

Culture grown anaerobically at 30°C to OD_{660} of 0.25 were treated for 15 min at 30°C with 4 μg/ml of mitomycin C (Kyowa Hakko Kogyo Ltd.; Ogata *et al.*, 1972). Excess mitomycin C was removed by centrifugation at 9000 × g for 5 min. The harvested organisms were resuspended in a TYA medium to an OD_{660} of 0.25, and the culture was incubated in the usual manner with monitoring of OD_{660} being made at 30- to 60-min intervals.

Separation of clostocin O and giant phage tails

The lysates of clostocin O were purified and concentrated by two cycles of the differential centrifugation as described previously (Ogata *et al.*, 1969). The clostocin O pellets were suspended in 0.1 M ammonium acetate buffer (pH 6.0). Then, clostocin O and giant tail particles were separated by cesium chloride gradient centrifugation according to Jones *et al.* (1974).

Assay of bacteriocin activity

The bacteriocin activity was assayed by the spot test using the separated specimens on solid cultures (Ogata *et al.*, 1972).

Electron microscopy

The clostocin O or phage suspension was mixed with an equal volume of 1 to 2% (w/v) potassium phosphotungstate (pH 6.0) or uranyl acetate (pH 6.0) and placed on grids coated with collogion-carbon. Electron micrographs were taken by a JEM-100B

electron microscope at 80 kV or a Philips EM 300 electron microscope at 60 kV. Also, the samples made directly from a single plaque or overlapping plaques were directly suspended in potassium phosphotungstate, as described previously (Ogata *et al.*, 1981).

RESULTS AND DISCUSSION

Structure of giant phage tail particles found in the mitomycin C–induced lysate with clostocin O and in the lysate of phage HM 3

The lysate of strain N1–4 induced by mitomycin C had two kinds of phage taillike particles. The first of these was the main product of the lysate, phage taillike bacteriocin, clostocin O with the extended sheath (approximately 125×22 nm) and the contracted sheath (approximately 80×27 nm or 60×27 nm): the size of contracted sheath was not

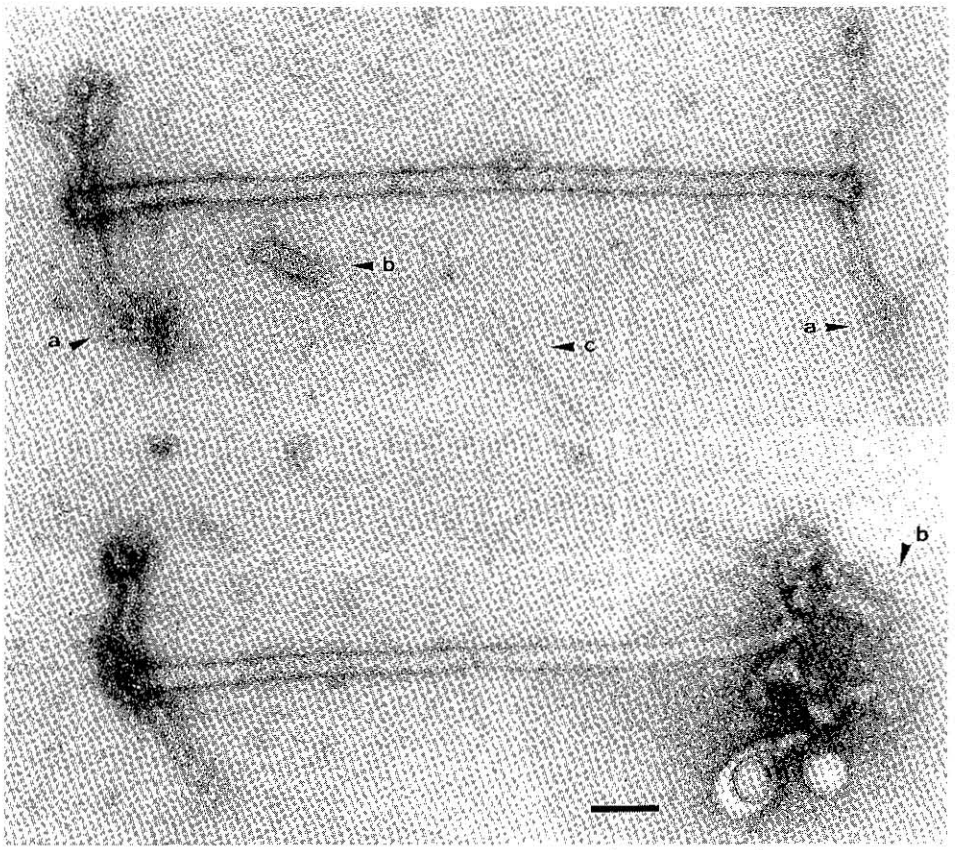

Fig. 1. Twin extended X particles.
Twin particles of X with two or three helical fibers are seen. Arrows a show the structures resembling suckers or funnels. Arrows b show contracted clostocin O and arrow c shows a part of host flagellum.
PTA was used for negative contrasting. Bar shows 100 nm.

uniformed in electron microscopy, it may be caused by the properties of clostocin O genome or by the artificial degradation of prepared clostocin O (Ogata *et al.*, 1972). Their morphology was similar to the tails of contractile phages such as phage T even of *E. coli* and phage HM 3 of the strain used in this paper and phage taillike bacteriocins such as pyocin of *Pseudomonas aeruginosa* (Bradley, 1967; Kageyama, 1964), carotoviocin of *Erwinia carotovora* (Itoh *et al.*, 1978) and xenorhabdicin of *Xenorhabdus nematophilus* (Thaler *et al.*, 1995). Clostocin O had bacteriocidal and bacteriolytic activity against some strains of *Clostridium* species (Kato *et al.*, 1976a; 1976b; 1977;

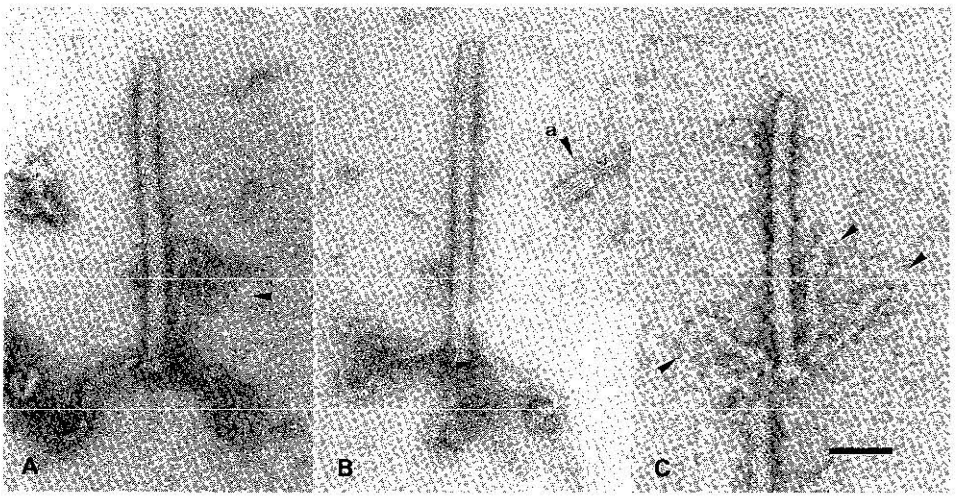

Fig. 2. Single extended X particles.
Single X particles with three helical fibers are seen. Arrows a show contracted clostocin O. The other arrows show the structures resembling suckers or funnels.
PTA was used. Bar shows 100 nm.

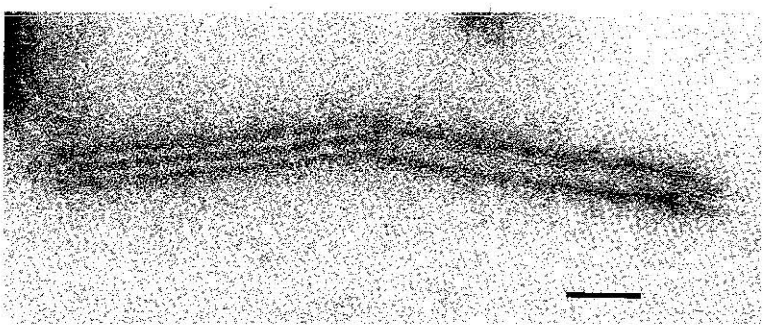

Fig. 3. Twin contracted X particles with hollow sheath or tail core. Contracted X with hollow sheath (left) and tail core (right). Uranyl acetate was used. Bar shows 100 nm.

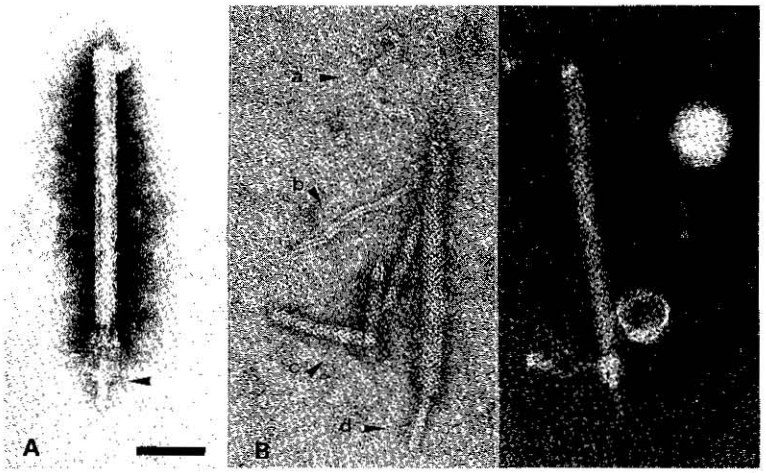

Fig. 4. Contracted X particles with or without fibers.
A: Tail core (arrow) arises from contracted X.
B: Long detached tail core of X is indicated by arrow b; Detached single helical fiber is shown by arrow a; Arrow d also shows tail core; Three extended clostocin O are shown by arrow c.
C: Contracted X with a helical fiber (arrow c) which is on the point of detaching; Arrows a and b show phage HM 3 and its empty head, respectively.
PTA was used. Bar shows 100 nm.

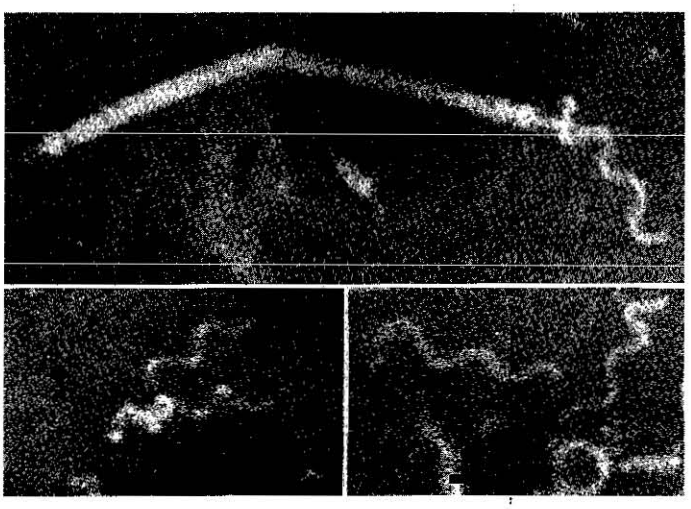

Fig. 5. Detached base plate and detached helical fiber of X particle.
A: Contracted X with base plate and a fiber (arrow a); Arrow b shows a contracted sheath of phage HM 3.
B: Detached plate with three fibers.
C: Two detached single fibers are seen together with ghost particle of phage HM 3 (arrow).
PTA was used. Bar shows 100 nm.

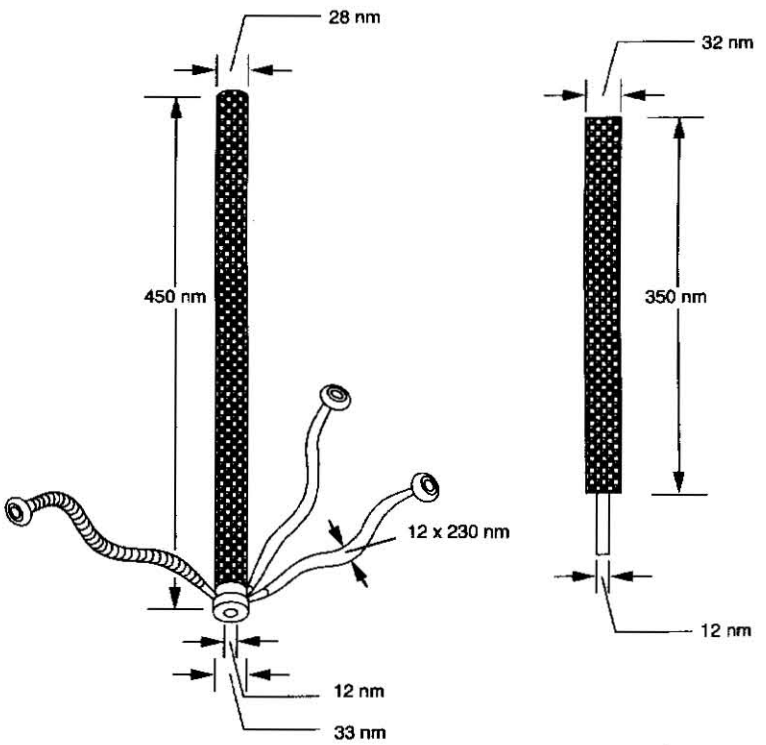

Fig. 6. Schematic diagrams of X particle. Left, extended X; Right, Contracted X.

Ogata *et al.*, 1972; 1976). The detailed structures and properties of clostocin O had been reported (Ogata *et al.*, 1972; 1978).

The second phage taillike rods, lesser products of the lysate, were large particles with a peculiar structure, as shown in Figs. 1 to 5. The structure of particles was shown schematically in Fig. 6. These giant phage tails, named X particles, were also produced on the occasion of phage HM 3 infection: HM3 had an isomeric head of 77 nm in diameter and a contractile tail with a sheath that measured 90×15 nm when extended and 42×21 nm when contracted (Ogata *et al.*, 1969). However, X particles were extremely in noninduced and phage-uninfected cultures. No bacteriocin activity has been found on X particles.

The extended X rods consisting of striated sheaths were about 450 nm in length and about 28 nm in width (Figs. 1, 2). At the distal end of the rod was a base plate, the diameter of which was about 33 nm. Three powerful wavy, helical or twisting whiplike fibers, composed of individual subunits, attached to the end plate or branched off from it. Two rods joined frequently each other at their tops (Figs. 1, 3, 5A). The X particle was very similar to the tail of phage Bace-11 of *Bacillus cereus*, as shown in Fig. 7: tails measured 485×20 nm in the extended state (Ackermann *et al.*, 1995).

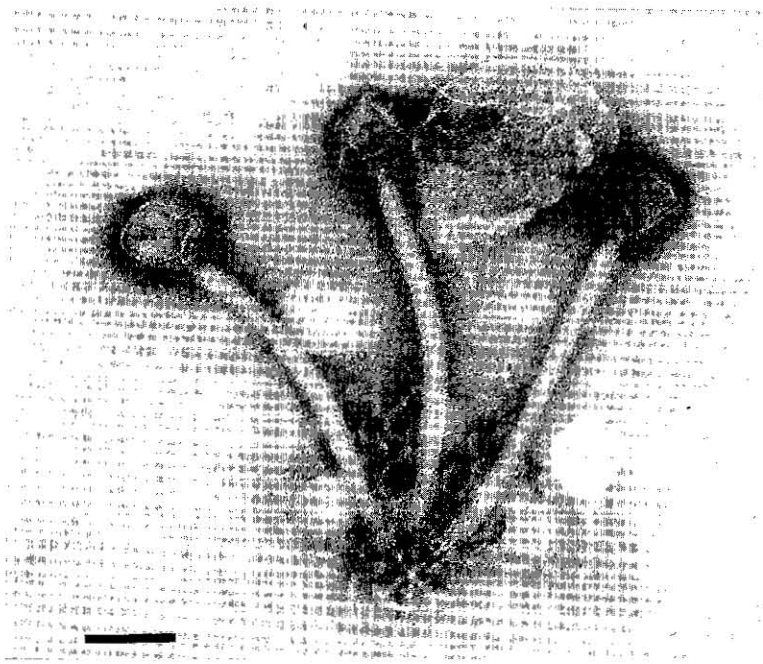

Fig. 7. Phage Bace–11 of *Bacillus cereus*. PTA was used. Bar shows 100 nm.

The contractile rods of X particles were also seen, as shown in Figs. 3, 4 and 5A. The sheath was estimated to be 350 nm in length and about 32 nm in width: contracted sheaths of phage Bace–11 measured 370×27 nm (Ackermann *et al.*, 1995). By the contraction or disruption, the base plate with the helical fibers detaches from the sheath, and a tail core arises. The tail core of X particle is about 12 nm in width and is surrounded by the sheath (Figs. 3 and 4). The hollow sheaths, which do not hold the tail core, also seen in Fig. 3. However, no heads or headlike particles were seen. It is therefore said that X particles belong to a category of defective phages or phagelike particles.

On the partial disrupted rod in Fig. 4C and 5A, a single fiber attached to the plate. The plate seemed to easily detach from the sheath. Detached plate with three fibers was observed (Figs. 5B). Each fiber ended in special structures resembling suckers or funnels (arrows in Figs. 1, 2), consisting of subunits which can be distinguished two or three–dimensionally. Many detached single fibers were also observed (Figs. 4B, 5C). They seemed to easily detach from the plates. The suckerlike structures seemed to be liable on the detached single fibers and the contracted or disrupted rods. The fiber was approximately 230×12 nm.

The powerful helical tail fibers have been observed to be associated with the tails of phages PBS1 (Ackermann and DuBow, 1987b; Eiserling, 1967) and AR9 (Belyaeva and Azizbekyan, 1968) of *Bacillus subtilis*, of phage Bace–11 described above, of phage X of

the enterobacteria (Ackermann and DuBow, 1987d; Meynell, 1961), and phage taillike particles of *Clostridium sporogenes* and *Clostridium innocuum* (Nieves *et al.*, 1981). In particular, the tail fibers of X particles exhibit a resemblance to the tail fibers of phages PBS1, AR9 and Bace–11 in the morphological structures and number, but with some difference in the size (230×12 nm for X particle, 220–230×8 nm for Bace–11 and 125×8 nm for PBS1). The other dimensions of the tail of phage PBS1 are listed for comparison with those of X particle: uncontracted sheath 200×22 nm and tail core 9 nm in width. The wavy, whiplike tail fibers of these phages seems to play a role on the infection toward the host bacterium. The conspicuous, long wavy tail fibers are wrapped around the flagellum of the host bacteria, on which the receptor sites for the phage adsorption are located (Ackermann and DuBow, 1987a; Raimondo *et al.*, 1968). A similar manner for the adsorption to the host should have be done by a progenitor of X particle, as *Clostridium* bacteria are flagellated and motile.

REFERENCES

Ackermann, H. –W., and M. S. DuBow. 1987a. Viruses of prokaryotes, Vol I, CRC Press, Florida.

Ackermann, H. –W., and M. S. DuBow. 1987b. Viruses of prokaryotes, Vol II, CRC Press, Florida, p72–81.

Ackermann, H. –W., and M. S. DuBow. 1987c. Viruses of prokaryotes, Vol II, CRC Press, Florida, p82–84.

Ackermann, H. –W., and M. S. DuBow. 1987d. Viruses of prokaryotes, Vol II, CRC Press, Florida, p85–100.

Ackermann, H. –M., S. Yoshino, and S. Ogata. 1995. A *Bacillus* phage that is a living fossil, Can. J. Microbiol., **41**, 294–297.

Belyaeva, N. N., and R. R. Azizbekyan. 1968. Fine structure of new *Bacillus subtilis* phage AR9 with complex morphology. Virology, **34**: 176–179.

Bradley, D. E. 1967. Ultrastructure of bacteriophages and bacteriocins. Bacteriol. Rev., **31**: 230–314.

Eiserling, F. A. 1967. The structure of *Bacillus subtilis* bacteriophage PBS1. J. Ultrastruct. Res., **17**: 342–347.

Itoh, Y., K. Izaki, and H. Takahashi. 1978. Purification and characterization of a bacteriocin from *Erwinia carotovora*. J. Gen. Appl. Microbiol., **24**: 27–39.

Jones, L. A., M. L. Hahn, and A. R. Taylor. 1974. Incomplete and aberrant particles of actinophage MSP2 from lysates of *Streptomyces venezuelae*. J. Virol., **14**: 493–502.

Kato, F., S. Ogata and M. Hongo. 1976a. Lytic action of inducible bacteriocin clostocin O. Agric. Biol. Chem., **40**: 1101–1105.

Kato, F., S. Ogata and M. Hongo. 1976b. Killing activity of clostocin O. Agric. Biol. Chem., **40**: 1107–1111.

Kato, F., S. Ogata and M. Hongo. 1977. Action of an inducible bacteriocin clostocin O on the macromolecular synthesis of *Clostridium saccharoperbutylacetonicum*. Agric. Biol. Chem., **41**: 1883–1888.

Meynell, E. W. 1961. A phage ϕX, which attacks motile bacteria. J. General. Microbiol., **25**: 253–290.

Nieves, B. M., F. Gil and F. J. Castillo. 1981. Growth inhibition activity and bacteriophage and bacteriocinlike particles associated with different species of *Clostridium*. Can. J. Microbiol., **27**: 216–225.

Ogata, S. 1980. Bacteriophage contamination in industrial process. Biotechnol. Bioengin., **22** (suppl.1): 177–193.

Ogata, S., and M. Hongo. 1979. Bacteriophages of the genus *Clostridium*. Advances in Appl. Microbiol., **22**: 241–273.

Ogata, S., F. Kato and M. Hongo. 1976. Study on adsorption of inducible bacteriocin clostocin O toward sensitive bacteria. Agric. Biol. Chem., **40**: 1093–1099.

Ogata, S., F. Kato, M. Miyanaga, S. Hayashida and M. Hongo. 1978. Purification and some properties of an inducible bacteriocin, clostocin O, of *Clostridium saccharoperbutylacetonicum*. J. Gen. Appl. Microbiol., **24**: 223–232.

Ogata, S., O. Mihara, Y. Ikeda and M. Hongo. 1972. Inducible phage tail-like particles of *Clostridium saccharoperbutylacetonicum* and its related strains. Agric. Biol. Chem., **36**: 1413–1421.

Ogata, S., N. Nagao, Z. Hidaka and M. Hongo. 1969. Bacteriophages of *Clostridium saccharoperbutylacetonicum*. Part XII. The structure of phage HM 2. Agric. Biol. Chem., **33**: 1541–1552.

Ogata, S, S. Yoshino, H. Suenaga, K. Aoyama, N. Kitajima and S. Hayashida. 1981. Specific lysogenicity in *Streptomyces azureus*. Appl. Environ. Microbiol., **42**: 135–141.

Raimondo, L. M., N. P. Lundh and R. J. Martinez. 1968. Primary adsorption site of phage PBS1: the flagellum of *Bacillus subtilis*. J. Virol., **2**: 256–264.

Schade, S. Z., J. Adler and J. Ris. 1967. How bacteriophage X attacks motile bacteria. J. Virol., **1**: 599–605.

Thaler, J.-O., S. Baghdiguian, and N. E. Boemare. 1995. Purification and characterization of *Xenorhabodus nematophilus*. Appl. Environ. Microbiol., **61**: 2049–2052.

Thkonenko, A. S. 1970. Ultrastructure of bacterial viruses. Plenum Press, New York.