*Symbiosis*, 22 (1997) 205–215
Balaban, Philadelphia/Rehovot


# New Antimicrobial Barriers Produced by *Xenorhabdus* spp. and *Photorhabdus* spp. to Secure the Monoxenic Development of Entomopathogenic Nematodes

J.-O. THALER, M.-H. BOYER-GIGLIO, and N.E. BOEMARE*
*Laboratoire de Pathologie comparée, INRA-CNRS URA 2209, CP 101, Université Montpellier 2, Sciences et Techniques, 34095 Montpellier Cedex 05, Tel. +33-467143740, Fax. +33-467144679, E-mail. boemare@ensam.inra.fr*

Received September 9, 1996; Accepted September 26, 1996

## Abstract

Bacteriocins occur naturally in low quantities in both phases of *Xenorhabdus* spp. as characterized by electron microscopical negative staining of culture supernatants and in vitro antibacterial activity. In both phases of most of *Xenorhabdus* cultures, induction by mitomycin C or high temperature resulted in a massive production of bacteriocins with few entire phage particles; such treatments cause a total lysis of the culture and the death of the producer cells. Phage DNA purified from *Xenorhabdus* strains hybridized to several fragments of chromosomal DNA restricted fragments, confirming that the phage genome is incorporated into the bacterial chromosome. Consequently *Xenorhabdus* strains are lysogenic, bearing lethal genes as a prophage which is transmitted during cellular divisions and becomes lytic only after induction. Similar induction experiments with *Photorhabdus* cultures failed to show a greater production of bacteriocins than the spontaneous production observed in supernatants of non induced cultures. At this stage phages were not yet shown to be produced by *Photorhabdus* strains. Conditions of the induction of a total lysis in *Photorhabdus* cultures have not been yet discovered. However, in the spontaneously lysed cells of *Photorhabdus*, image processing of electron microscopic preparations shows that protoplasmic rods in longitudinal section and hexagonal aggregates in transversal section have a morphological homology with purified bacteriocins from *Xenorhabdus*. These so-called "lattice structures", previously interpreted as "photosomes" for *Photorhabdus* cells (Boemare et al., 1983), are in fact the early stages of in situ production of bacteriocins for a weak proportion of the cellular population in

*The author to whom correspondence should be sent.

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symbiont cultures (Baghdiguian et al., 1993). Bacteriocins from several *Xenorhabdus* strains are bactericidal for closely related bacteria such as other *Xenorhabdus* spp., *Photorhabdus* spp. and some *Proteus* spp. The synergistic effects of these bacteriocins produced by both phases, and the previous identified water-soluble antibiotics only produced by *Xenorhabdus* phases I (Akhurst, 1982), participate strongly for conditions observed during the parasitic development of the host nematode into insect cadavers, which favor the *Xenorhabdus* or *Photorhabdus* as the dominant culture.

Keywords: Lysogeny, bacteriophages, bacteriocins, bacteriolysis

## 1. Introduction

The genus *Xenorhabdus* (Thomas and Poinar, 1979) and the genus *Photorhabdus* (Boemare et al., 1993) share common properties probably linked with their symbiotic status. Among these properties, phase variation and antimicrobial activity are quite well documented this decade. Phase I colonies produce agar-diffusible antibiotic substances (Akhurst, 1982), never secreted by phase II except with some strains of *X. poinarii* (Akhurst, 1986). Those identified to date are indole derivatives (Paul et al., 1981), trans-stilbene derivatives (Richardson et al., 1988), xenorhabdins (McInerney et al., 1991a) and xenocoumacins (McInerney et al., 1991b). But during this last decade many controversial observations seem to indicate that other antimicrobial agents are produced by both phases. They are difficult to evidence, either because they are not diffusible in agar, and/or because their action accumulates concurrently with the previous identified antimicrobial molecules hiding a cumulative effect. Among these possible new antimicrobial agents the phages, and particularly the bacteriocins, play an important part. The term "bacteriocin" was coined by Jacob et al. (1953) for proteinaceous antibacterial agents which were synthesized by bacteria and required specific receptors (Ackermann and Dubow, 1987). Among several types, one group includes phage tail-like particles consisting of contractile tails also called particulate bacteriocins, lethal phages or defective phages (Kageyama, 1975).

Given this background, our laboratory has been concerned with the possibility of lysogeny in *Xenorhabdus* spp. and *Photorhabdus* spp. in order to find a more general cause of unexplained spontaneous lysis in cultures of *Xenorhabdus* spp. In several experiments we have demonstrated the occurrence of a lysogeny and a bacteriocinogeny in *Xenorhabdus* spp. (Boemare et al., 1992). Steps of the bacteriocin synthesis in *Xenorhabdus* and *Photorhabdus* cells have been described (Baghdiguian et al., 1993) and the possible ecological role of these antimicrobial agents for the nematode bacterium complexes has been discussed (Boemare et al., 1994). In this report the status of this research is summarized and updated.

## 2. Methods and Results

*Induction of lysis in Xenorhabdus lysogenic strains and analysis of the produced elements*

Two major treatments were used to induce a *Xenorhabdus* culture lysis. The first used mitomycin C (0.5 to 1 µg/ml, depending on strains) added to a Luria broth (LB) culture of each *Xenorhabdus* phase at logarithmic growth ($A_{600}$ = 0.5). The second was an heating (45°C, 40 min) of LB broth when culture raised the middle of logarithmic growth ($A_{600}$ = 0.5), followed by an overnight subsequent incubation at 28°C (Boemare et al., 1992). After heating or mitomycin C treatment, both phase I and II cultures lysed 3–5 h later.

*Ultrastructural examination of the produced components*

Lysates were examined on transmission electron microscope by negative staining with 1% phosphotungstate. For many *Xenorhabdus* strains, several structural components were characterized such as rigid phage tail-like particles, empty phage heads, and few intact phage particles (Boemare et al., 1992). The rigid phage tail-like particles presented a large variety of forms, including headless rods with extended striated sheaths ca. 170 nm in length possessing a baseplate to which fibers with adhesive extremities were attached (caudal fibers), contracted sheaths revealing an inner core, segments of empty contracted sheaths, and loose cores (Fig. 1). The morphological analysis of the pictures by a mathematical approach raised three important points: (i) the hexagonal section of the sheath, each side 4.2 nm sized giving an average diameter ca. 8.4 nm, (ii) the helical organization of the sheath, and (iii) the six fold symmetry forming this helical organization (Baghdiguian et al., 1993). These morphological characteristics were basically those of rigid phage tails. At the contrary the few phages, observed in mixture with the rigid phage tail-like particles, had flexible tails (Fig. 1).

*Purification of the Xenorhabdus nematophilus phage tail-like particles*

Purification of the phage tail-like particles of *X. nematophilus* strain F1 was previously described in details (Thaler, 1994; Thaler et al., 1995). Lysates obtained by the previous treatments, were first treated with DNase and RNase, the protein contents were precipitated, and pellets were deposited on a 10–40% (wt/vol) sucrose density gradient. An opalescent band at around 15% sucrose gradient was collected, dialyzed, and chromatographied. One exclusion peak and one elution peak were generated. Electron microscopy of DEAE chromatographic elution peak aliquots showed only particles with extended

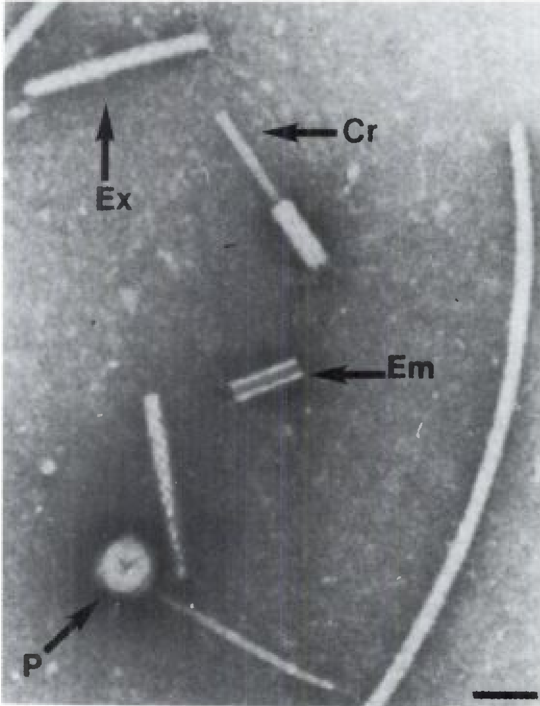


Figure 1. Supernatant of purified lysate of *X. nematophilus* F1 culture in LB broth after induction by mitomycin C. Ex = xenorhabdicin with rigid extended sheath, Cr = xenorhabdicin with rigid contracted sheath, Em = empty sheath of xenorhabdicin, P = Phage with flexible tail. Electron micrograph of 1% phosphotungstate negative staining. Scale bar = 50 nm.

sheaths, while the exclusion peak aliquot revealed empty phage head particles.

The particles with extended sheaths were composed of two major sub-units and two minor sub-units. Molecular weights were estimated from their apparent mobility in SDS-PAGE. The two first were 43 and 20 KDa, corresponding to the sheath and the inner core, and of the two second were about 67 and 54 KDa, corresponding to structural proteins of the caudal fibers.

*Characterization of the phages*

Three bands were identified by electrophoresis of a sample from the previous exclusion peak: one ca. 40 corresponding to the major component of the phage capsomers and two minor ca. 50 and 34 KDa. These elements are the

protein subunits of the empty phage heads (Thaler et al., 1995). The DEAE chromatography exclusion peak produced no DNA band, confirming that the previous purification isolated only empty phage head particles.

On the contrary, DNA extraction by method of Zyskind and Bernstein (1989) from lysates of *X. nematophilus*, *X. bovienii* and *X. beddingii* revealed a band above the 23 Kb marker. This linear viral DNA band was well distinguished from plasmid and chromosomal DNA of the producer strain. This phage DNA was used as a probe to hybridize to restriction enzyme digested genomic DNAs of *Xenorhabdus* producer strains, and of closely related species as control, and transferred onto a nitrocellulose filter by southern blotting. Strong hybridizations were shown with genomic restricted bands of only the producer strain. This suggests an occurrence of a specific prophage on genomic DNA of each *Xenorhabdus* (Boemare et al., 1992). On the other hand there was neither homology to plasmid bands (Leclerc and Boemare, 1991) of producer strain, nor to digested chromosomal DNA fragments of other closely related species used as a control. Consequently a prophage is incorporated into the chromosome of the bacterial host.

### *In vitro bactericidal activity of Xenorhabdus phage tail-like particles*

Bactericidal activity of *Xenorhabdus* phage tail-like particles at different steps of purification was tested by a logarithmic serial dilutions method. This in vitro method used culture lysates, sterilized by passage through a 0.45 µm pore size Millipore™ filter, or purified suspensions, which were tested by spotting onto lawns of the sensitive strain. Several indicator strains (Enterobacteriaceae, Pseudomonadaceae, Vibrionaceae and Bacillaceae), from a logarithmic-period growth ($A_{600}$ = 0.5) culture on LB, were mixed (2% vol/vol) into 5 ml of nutrient agar 0.6% (wt/vol) and spread onto nutritive agar Petri dishes. The ten fold serial dilutions of the phage tail-like particle suspensions were spotted onto these plates. A clear zone on the bacterial lawn at the drop location after incubation at 28°C for 24 h was interpreted as an inhibition of the indicator bacterium.

The bactericidal specific activity of *X. nematophilus* phage tail-like particles (Table 1), increased gradually as its concentration during the purification procedure described above. *Morganella morganii, Proteus vulgaris, Photorhabdus luminescens* and *X. beddingii* were highly sensitive to the suspensions prepared from the two phases. Non-induced cultures of *Xenorhabdus* were immune to the purified suspensions of phage tail-like particles obtained from their own strain. Sensitivity to the suspensions was eliminated by treating with pronase E (5 U/ml) or by heating (65°C, 30 min). This indicated that the sensitivity of *P. morganii, P. vulgaris, P. luminescens*

Table 1. Antibiotic activity of various dilutions of bacteriocin suspension purified from induced cultures of *X. nematophilus* A24/1 and A24/2

| Inhibition[a] by Indicator strains | Control[b] | | Bacteriocin suspension[c] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $10^{0}$ | $10^{-1}$ | $10^{0}$ | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ |
| *Citrobacter freundii* | – | – | – | – | – | – | – | – |
| *Enterobacter cloacae* | – | – | – | – | – | – | – | – |
| *Erwinia chrysanthemi* | – | – | – | – | – | – | – | – |
| *Klebsiella pneumoniae* | – | – | – | – | – | – | – | – |
| *Salmonella typhimurium* | – | – | – | – | – | – | – | – |
| *Serratia marcescens* | – | – | – | – | – | – | – | – |
| *Yersinia enterolitica* | – | – | – | – | – | – | – | – |
| *Pseudomonas aeruginosa* | – | – | – | – | – | – | – | – |
| *Pseudomonas testosteroni* | – | – | – | – | – | – | – | – |
| *Micrococcus luteus* | – | – | – | – | – | – | – | – |
| *Micrococcus roseus* | – | – | – | – | – | – | – | – |
| *Streptococcus epidermidis* | – | – | – | – | – | – | – | – |
| *Streptococcus faecalis* | – | – | – | – | – | – | – | – |
| *Bacillus cereus* | – | – | – | – | – | – | – | – |
| *Bacillus megaterium* | – | – | – | – | – | – | – | – |
| *Bacillus subtilis* | – | – | – | – | – | – | – | – |
| *Bacillus thuringiensis* | – | – | – | – | – | – | – | – |
| *Escherichia coli* | – | – | – | – | – | – | – | – |
| *Proteus mirabilis* | – | – | – | – | – | – | – | – |
| *Morganella morganii* | + | – | + | + | + | + | – | – |
| *Proteus vulgaris* | + | – | + | + | + | + | – | – |
| *Photorhabdus luminescens* | | | | | | | | |
| K80/1 | + | – | + | + | + | + | + | – |
| K80/2 | + | – | + | + | + | + | + | – |
| *Xenorhabdus beddingii* | | | | | | | | |
| Q58/1 | + | – | + | + | + | + | + | + |
| Q58/2 | + | – | + | + | + | + | + | – |
| *Xenorhabdus nematophilus* | | | | | | | | |
| F1/1 | – | – | – | – | – | – | – | – |
| F1/2 | – | – | – | – | – | – | – | – |
| A24/1 | – | – | – | – | – | – | – | – |
| A24/2 | – | – | – | – | – | – | – | – |

[a]Inhibition was scored as + or –; + = clear zone at the droplet site; – = no clear zone. [b]Non-induced cultures of *X. nematophilus* A24 ($A_{600}$ = 0.5) were centrifuged and the filter-sterilized supernatant used as the control. The control supernatant was diluted to $10^{-1}$ with phosphate buffer to assess the residual bacteriocin activity. [c]The bacteriocin suspension was diluted to $10^{-5}$ with phosphate buffer to assess the strength of bacteriocin activity. (Table reprinted from Boemare et al., 1992).

and *X. beddingii* was due to the proteinaceous phage tail-like particles in the purified suspensions. Bactericidal specific activity increased approximately 450-fold from the previous elution peak of chromatography while no bactericidal activity was found in the exclusion peak (Thaler et al., 1995).

Consequently, electron microscopic evidence, biochemical properties, antimicrobial activity against closely related bacteria, and sensitivity to protease and heat treatments, showed that the phage tail-like particles were bacteriocins. These bacteriocins were named xenorhabdicins (Thaler et al., 1995).

*In situ synthesis of bacteriocin elements in Xenorhabdus cells*

For cytomorphological studies, bacterial cell pellets were fixed with 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, and sections were contrasted with uranyl acetate and lead citrate as described in Baghdiguian et al. (1993). In ultrathin sections of *Xenorhabdus* cells from cultures induced after 2 hours, numerous cells displayed densely packed rods as longitudinal bands and hexagonal aggregates. Hexagons were at about 10.0 nm from center to center, similar to the hexagon size of bacteriocin sections. Morphogenesis of these bacteriocins proceeded helically from the center, where the bacteriocins appeared transversally as hexagons, to the periphery, where progressively they were in length section. But they appeared also longitudinally on other sections as a palisade forming longitudinal bands and lines. Occasionally some altered cells were observed at this stage. Then, between 4 and 6 hours after the induction, an elaborate extensive array of bacteriocin matrices was progressively seen in many altered cells where the cell wall was in many points disrupted. Finally bacteriocins were in cellular ghosts and were released outside due to disruption of cellular membranes.

*Occurrence of bacteriocins in Photorhabdus*

When cultures in stationary growth of *Photorhabdus* were stained by 1% phosphotungstate, electron microscopy examinations showed few bacteriocin particles neighbouring many healthy cells. At the present time, no suitable procedure of lysis induction for these species has been developed. However, a low level of bacteriocins was commonly observed between normal bacterial cells. In ultrathin sections of the few altered cells observed among an apparent healthy population, bacteriocin particles were recognized such as piles of superimposed layers of sheaths similar in length to the previous *X. nematophilus* bacteriocins (150–200 nm). Bacteriocins were also observed agglomerated in packages as the result of the residual contents of a cell

disruption. All these elements were recognized for several strains of *P. luminescens* strongly indicating natural occurrence of a synthesis of bacteriocins in *Photorhabdus* spp. (Baghdiguian et al., 1993).

## 3. Discussion and Conclusion

The occurrence of temperate bacteriophage in both phases of *Xenorhabdus* indicates that phase variation is not a mechanism for escaping the lytic activity of the phage as previously suggested (Poinar et al., 1989). Hybridization of DNA phage on the chromosome of its bacterial host and inducible bacteriolysis by mutagenic agents, are arguments to assume that a lysogeny occurs in *Xenorhabdus* spp. The lysogeny in *Xenorhabdus* spp. is a general phenomenon but, for each strain and species, conditions of the lysis induction need to be defined.

Xenorhabdicins of *Xenorhabdus* belong to the group of so-called phage tail like particles or defective phages which are bactericidal (Ackermann and Dubow, 1987). They are very similar to the R-type pyocins of *P. aeruginosa* with a contractile sheath surrounding a core, a baseplate and six fibers considered to be the apparatus for adsorption to the receptors on sensitive cells (Bradley, 1967; Kageyama et al., 1979). Most of these phage tails like numerous other phage tails (Ackermann and Dubow, 1987) are helical with six fold symmetry. Image processing allows us to demonstrate this six-fold symmetry and an hexagonal section of the xenorhabdicin tail (Baghdiguian et al., 1993). They have a high molecular weight, sediment easily and appear as phage tail like particles in the electron microscope as do numbers of other described contractile tails (Echandi and Moyer, 1979). While bacteriocins have been described in a widespread distribution of bacterial genera, in *Xenorhabdus* they seem to be very similar to those associated with lysogenic strains of Enterobacteriaceae and Pseudomonadaceae (Echandi et al., 1979; Ito et al., 1970; Kageyama, 1975).

Lysogeny and bacteriocinogeny are often common characters, and the production of these agents is inducible by UV irradiation, mitomycin C, $H_2O_2$, or temperature treatments (Bradley, 1967; Ito et al., 1970; Kageyama, 1975). Our studies have shown that bacteriocins are widespread in *Xenorhabdus*. Occurrence of this phenomenon may explain frequent disruptions in maintenance of *Xenorhabdus* samples (e.g. when temperature conditions are not well controlled). The studies presented here reveal a class of agents with an activity against a limited range of bacteria, including closely related species. Bacteriocins have a much narrower spectrum of antimicrobial activity than most chemical antimicrobials (Bradley 1967; Kageyama 1975; Mayr-Harting et al.,

1972). So in addition to antimicrobial chemicals production by Xenorhabdus spp phases I, effective against a large scale of microorganisms, there is an antimicrobial proteinic production by both phases of *Xenorhabdus* which are the bacteriocins. These bacteriocins also prevent contamination of foreign microorganisms, but mainly the contamination of closely related microorganisms such as all the other *Xenorhabdus* spp.

When one *Steinernema* sp. and one *Heterorhabditis* sp. co-infected simultaneously an insect the dominance of the *Steinernema* is observed (Alatorre-Rosas and Kaya, 1991). It is supposed that the *Heterorhabditis* may take longer to release its bacterial symbiont, giving a competitive advantage to the co-infective *Steinernema*. Once a development is initiated toxins or other byproducts from the *Xenorhabdus* may have prevented the development of *H. bacteriophora*. The dominance of one *Xenorhabdus* species in an insect host that determines which of any co-infecting nematode species can survive and reproduce (Alatorre-Rosas and Kaya, 1991) is very likely mediated by the xenorhabdicins. The first released bacteria may initiate the spontaneous production of bacteriocins reported here which should be able to kill other related bacteria. Then the nematode species devoid of its symbiont is not able to reproduce in the insect cadaver. Bacteriocins probably participate strongly to prevent any foreign candidate to combine with the normal host nematode of their producer bacteria (Boemare et al., 1994).

## REFERENCES


Ackermann, H.-W. and Dubow, M.S. 1987. *Viruses of Prokaryotes*. Vol. I *General Properties of Bacteriophages*. CRC Press, Boca Raton, USA, 202 pp.

Alatorre-Rosas, R. and Kaya, H.K. 1991. Interaction between two entomopathogenic nematode species in the same host. *Journal of Invertebrate Pathology* **57**: 1–6.

Akhurst, R.J. 1982. Antibiotic activity of *Xenorhabdus* spp., bacteria symbiotically associated with insect pathogenic nematodes of the families *Heterorhabditidae* and *Steinernematidae*. *Journal of General Microbiology* **128**: 3061–3065.

Akhurst, R.J. 1986. *Xenorhabdus nematophilus* subsp. *poinarii*: its interaction with insect pathogenic nematodes. *Systematic and Applied Microbiology* **8**: 142–147.

Baghdiguian, S., Boyer-Giglio, M.-H., Thaler, J.-O., Bonnot, G., and Boemare, N.E. 1993. Bacteriocinogenesis in cells of *Xenorhabdus nematophilus* and *Photorhabdus luminescens*: Enterobacteriaceae associated with entomophathogenic nematodes. *Biology of the Cell* **79**: 177–185.

Boemare, N.E. and Akhurst, R.J. 1988. Biochemical and physiological characterization of colony form variants in *Xenorhabdus* spp. (Enterobacteriaceae). *Journal of General Microbiology* **134**: 1835–1845.

Boemare, N.E., Akhurst, R.J., and Mourant, R.G. 1993. Deoxyribonucleic acid relatedness between *Xenorhabdus* spp. (Enterobacteriaceae), symbiotic bacteria of entomopathoge-

nic nematodes, with a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen.nov. *International Journal of Systematic Bacteriology* **43**: 249–255.

Boemare, N.E., Boyer-Giglio, M.-H., Thaler, J.-O., and Akhurst, R.J. 1994. The phages and bacteriocins of *Xenorhabdus* spp., symbiont of the nematodes *Steinernema* spp. and *Heterorhabditis* spp. In: Nematodes and the Biological Control of Insect Pests. R. Bedding, R. Akhurst and H. Kaya, eds. CSIRO, Melbourne, Australia, pp. 137–145.

Boemare, N.E., Boyer-Giglio, M.-H., Thaler, J.-O., Akhurst, R.J., and Brehélin, M. 1992. Lysogeny and bacteriocinogeny in *Xenorhabdus* spp., bacteria associated with entomopathogenic nematodes. *Applied and Environmental Microbiology* **58**: 3032–3037.

Boemare, N.E., Louis, C., and Kuhl, G. 1983. Etude ultrastructurale des cristaux chez *Xenorhabdus* spp., bactéries inféodées aux nématodes entomophages *Steinernematidae* et *Heterorhabditidae*. *Comptes-Rendus de la Société de Biologie* **177**:107–115.

Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry* **72**: 248-254.

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

Echandi, E. and Moyer, J.M. 1979. Production properties and morphology of bacteriocins from *Erwinia chrysantemi*. *Phytopathology* **69**: 1204–1207.

Ito, S., Kageyama, M., and Egami, F. 1970. Isolation and characterization of pyocins from several strains of *Pseudomonas aeruginosa*. *Journal of General Applied Microbiology* **16**: 205–214.

Jacob F., Lwoff, A., Siminovitch, A., and Wollman, E. 1953. Définition de quelques termes relatifs à la lysogénie. *Annales de l'Institut Pasteur (Paris)* **84**: 222.

Kageyama, M. 1975. Bacteriocins and bacteriophages. In: *Microbial Drug Resistance*. S. Mitsuhashi and H. Hashimoto, eds. Tokyo Univ. Press, Tokyo, Japan, pp. 291–305.

Kageyama, M., Shinomiya, T., Aihara, Y., and Kobayashi, M. 1979. Characterization of a bacteriophage related to R-type pyocins. *Journal of Virology* **32**: 951–957.

Leclerc, M.-C. and Boemare, N.E. 1991. Plasmid and phase variation in *Xenorhabdus* spp. *Applied and Environmental Microbiology* **57**: 2597–2601.

Mayr-Harting, A., Hedges, A.J., and Berkeley, R.C. 1972. Methods for studying bacteriocins. In: *Methods in Microbiology*. G. Morris and D. Ribons, eds. Academic Press, NY, pp. 315–422.

McInerney, B.V., Gregson, R.P., Lacey, M.J., Akhurst, R.J., Lyons, G.R., Rhodes, S.H., Smith, D.R.J., Engelhardt, L.M., and White, A.H. 1991. Biologically active metabolites from *Xenorhabdus* spp. Part I: Dithiopyrrolone derivatives with antibiotic activity. *Journal of Natural Products* **54**: 774–784.

McInerney, B.V., Taylor, W.C., Lacey, M.J., Akhurst, R.J., and Gregson, R.P. 1991. Biologically active metabolites from *Xenorhabdus* spp. Part II: Benzopyran-1-one derivatives with gastroprotective activity. *Journal of Natural Products* **54**: 785–795.

Paul, V.J., Frautschy, S., Fenical, W., and Nealson, K.H. 1981. Antibiotics in microbial ecology. Isolation and structure assignment of several new antibacterial compounds from the insect-symbiotic bacteria *Xenorhabdus* spp. *Journal of Chemical Ecology* **7**: 589–597.

Poinar, G.O., Hess, R.T., Lanier, W., Kinney S., and White J. 1989. Preliminary observations of a bacteriophage infecting *Xenorhabdus luminescens* (Enterobacteriaceae). *Experientia* **45**: 191–192.

Richardson, W.H., Schmidt, T.M., and Nealson, K.H. 1988. Identification of an anthraquinone pigment and a hydroxystilbene antibiotic from *Xenorhabdus luminescens. Applied and Environmental Microbiology* **54**: 1602–1605.

Thaler, J.-O. 1994. Purification and characterization of a particulate bacteriocin of *Xenorhabdus nematophilus* strain F1. Abstract of the VIth International Colloquium of Invertebrate Pathology & XXVIIth Annual Meeting of the Society for Invertebrate Pathology, August 28 – September 2, 1994, Montpellier, pp. 3–4.

Thaler, J.-O., Baghdiguian, S., and Boemare, N. 1995. Purification and characterization of nematophilicin, phage tail like bacteriocin, from the lysogenic strain F1 of *Xenorhabdus nematophilus. Applied and Environmental Microbiology* **61**: 2049–2052.

Thomas, G.M. and Poinar, G.O. 1979. *Xenorhabdus* gen. nov., a genus of entomo-pathogenic and nematophilic bacteria of the family Enterobacteriaceae. *International Journal of Systematic Bacteriology* **29**: 352–360.

Zyskind, J.W. and Bernstein, S.I. 1989. *Recombinant DNA Laboratory Manual.* Academic Press, NY.