

Full Length Research Paper

Nigerian phages: The first bacteriophages from Tropical Africa

Adeniran S. Koko^{1,3}, Hans W. Ackermann^{2*}, Monisola A. Taiwo¹ and Sunday A. Omilabu¹

¹Department of Botany and Microbiology, University of Lagos, Lagos, Nigeria.

²Félix d'Hérelle Reference Center for Bacterial Viruses, Department of Microbiology, Faculty of Medicine, Laval University, Québec, QC, G1K 7P4, Canada.

³Quest Diagnostics West Hills, CA 91304, CA, USA.

Accepted 03 March, 2019

Fifteen morphological bacteriophage types were observed in enrichment cultures of sewage, surface water and human feces collected in Lagos, Nigeria. All phages were tailed and belonged to the Myoviridae, Siphoviridae or Podoviridae families. Most phages could be assigned to well-known morphotypes. Ten of them corresponded to phages of enterobacteria or pseudomonads. Four morphotypes (ϕ KZ, T4 and χ , Jersey) prevailed in most samples. Most others occurred in traces only.

Key words: Bacteriophages, ecology, identification, morphotypes.

INTRODUCTION

Phage ecology has essentially been investigated in North America, Europe and the oceans. Research into phage ecology in other parts of the world is almost nonexistent. As a result the phage flora of vast geographic areas, namely most of South America, Africa, Australia, China, India, all of Middle America, the Middle East and Siberia remains essentially unknown. The only reports on phages from African environments are limited to tailed phages in the subsoil of the Moroccan and Tunisian Sahara (Prigent et al., 2005) and the Namibian desert (Prestel et al., 2008), RNA phages in sewage in South Africa (Grabow et al., 1993; Schaper et al., 2002), and unknown phages of nonidentified bacteria in Senegal (Bettarel et al., 2006). The Saharan phages were observed by electron microscopy, but not propagated or identified. The Namibian phages were observed by electron microscopy in bulk cultures, but they were not isolated and only one of the morphotypes present was identified. In addition, there are isolated reports of specific phages in various parts of the world, for example *Vibrio* phages from shrimp fisheries in Thailand (Pasharawipas et al., 2005), *Bacillus* phages from deep sea

wells in China (Liu et al., 2006), phages of particular actinomycetes in Australia (Kurtböke et al., 1993a, 1993b), and *Thermus* phages in Kamchatka hot springs (Yu et al., 2006).

This situation is deeply unsatisfactory from an ecological viewpoint. We intended to identify and diagnose our phages as precisely as possible. This is the first investigation of the bacteriophage flora in tropical Africa.

MATERIALS AND METHODS

We collected samples of sewage and surface water at the University of Lagos and in the Iddo area of the Lagos Lagoon, a natural lagoon of brackish water connected to the Atlantic Ocean through creeks. Nineteen water samples and one of human feces were enriched in Lagos, often three to four times in parallel, with common bacteria isolated from human wound and skin infections in Lagos hospitals (*Bacillus* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Xanthomonas* sp.). Enrichment cultures were filtered, but single plaque purification was not attempted. Samples were examined in Canada by one of us (H.-W.A.). Approximately 75 enrichment cultures of 1 ml each were received. Although all vials were intact and none had leaked during transportation, all were heavily contaminated by viable enterobacteria and pseudomonads. This contamination had clearly occurred in Nigeria before shipment. Samples were too small for filtration and were clarified by simple decantation during storage for 1 month. Supernatants were centrifuged at 25,000 g using a Beckman J2-21 centrifuge and a

*Corresponding author. E-mail: ackermann@mcb.ulaval.ca. Tel: +418 656-2131; 2558. Fax +418 656-7555.

Table 1. Bacteriophage observations by sample.

Sample	Bacteriophage morphotype								
	A41	φKZ	Lu11	T4	χ (chi)	Jersey	N4	T7	Others
1	*	+			+	+	+		φ29, 44AJHD
2	++	+		(+)	*		+		
3		+++		(+)	*				
4			++	*	*	*			
5	*	++			*	*		*	
6		+			++			*	
7		*			**	*			M1, S1
8			++	*	**	*		+	
9	*	++		+	+	++	*		
10		**		+	+	++			
11	*	+		*	++				
12	**	*		++		*		*	M1, S1
13	*	+		*		*			
14		+	+	*	*	++			121
15			+	*	+	+++			
Iddo			(+)	*		+	*		φ29
OPP1	*	*			*	*			
Berger				(+)	+				
Unilag	(+)				*	(+)			S2
Feces	*		+	*	*	(+)		*	T5, φ29

+++, ++, +, (+), *: Variable quantities of phages; +++, many; *, traces. M1, S1, S2: Novel morphotypes of Myoviridae (M) and Siphoviridae (S) families.

Table 2. Main dimensions of bacteriophage morphotypes.

Family	Type	Head, nm	Tail, nm
Myoviridae	YerA41	130	235 x 20
	φKZ	121	190 x 21
	Lu11	121	190 x 21
	121	116	115 x 17
	M1	133	156 x 17
	T4	111 x 78	113 x 16
Siphoviridae	χ (chi)	63	227 x 10
	Jersey	68	160 x 8
	T5	78	183 x 8
	S1	99	664 x 8
	S2	90	237 x 8
Podoviridae	N4	70	10
	T7	60	17 x 8
	φ29	54 x 42	36 x 12
	44JHD	45	35 x 12

JA-18.1 fixed-angle rotor and were washed twice with 0.1 M ammonium acetate (pH 7.0). Sediments were deposited on copper grids with carbon-coated Formvar films, stained with 2% potassium phosphotungstate (pH 7.0) and examined in a Philips EM 300 electron microscope. Magnification was calibrated by means of T4 phage tails (length 114 nm).

RESULTS AND DISCUSSION

Fifteen morphological bacteriophage types, listed roughly by family and size in Tables 1 and 2, were observed. All were tailed and had contractile, long and noncontractile or short tails. They were thus identified as members of the Myoviridae, Siphoviridae or Podoviridae families, respectively. Twelve morphotypes (shortly "types") corresponded to well-known phages described in earlier reviews or books to which the reader is referred (Ackermann and DuBow, 1987; Ackermann, 1987). Part of the corresponding phage groups (φKZ, T4, T7, N4, φ29 and AJHD) relate to phage genera or subfamilies defined by the International Committee on Taxonomy of Viruses or ICTV (Hendrix and Casjens, 2005; Lavigne et al., 2008; 2009). Ten types corresponded to known enterobacterial and *Pseudomonas* bacteriophage species and only two corresponded to species of *Bacillus* or *Staphylococcus* phages (podovirus types φ29 and 44AJHD, respectively). Most preparations were mixtures of 4 to 5, even 7 phage morphotypes (Table 1), suggesting that none of the bacteria used for enrichment had been pure. Most phage morphotypes could be related to well-known viruses with specific hosts. Four morphotypes were almost ubiquitous, namely : φKZ, T4, Jersey and χ (chi). Others occurred in traces only and were detected by intense scrutinizing only. This is not an

indication of their frequency or rarity in nature. No exact quantitation is possible here because the apparent frequency of phages in enrichment cultures depends on many variables (phage inoculum, host sensitivity, vitality of phages and nutrients), all of which may lead to overcrowding of some phages by others.

Part of this may have occurred during transportation since, as noted earlier, no sample was sterile on arrival in Canada. Finally, the very procedure of negative staining often leads to uneven distribution of particles and gathering of different particles in different places of electron microscopical grids. Three particle types (M1, S1, S2; myovirus 1, siphovirus 1 and 2) could not be attributed to known bacteriophages and may represent novel species. Type S1 is particularly interesting. Types M1 and S2 are morphologically unremarkable and not illustrated for lack of space.

Myoviridae

Morphotype A41 corresponds to a giant phage of this name, observed in *Yersinia enterocolitica* in Canada (Stevenson and Airdrie, 1984). It has so far not been found elsewhere. Types ϕ KZ and Lu11 correspond to giant *Pseudomonas* phages distinguished by the presence of long, curly tail fibers in the latter (Figure 2). Phages of type ϕ KZ were previously isolated in Denmark, Germany, Georgia, Russia and the U.S.A. Type Lu11 is known from the Philippines only (Krylov et al., 2007). The relative frequency of the ϕ KZ and Lu11 types is not surprising as representatives of these types multiply extremely well and may conceivably overcrowd other phages in mixed cultures. Enterobacterial phages of type 121 were first observed in Romania (Nacesco et al., 1969) and later in Canada by one of us (Ackermann and Nguyen, 1983). Type 121 is characterized by a large head and a relatively short tail. Type T4, easily recognized by its elongated head and a tail with six long tail fibers, seems to be truly ubiquitous and has been reported in many countries (Ackermann and Krisch, 1997) (Figures 1 to 4).

Type M1 does not, to our knowledge, correspond to any myovirus known. As types A41, ϕ KZ, Lu11 and 121, it has an unusually large head.

Siphoviridae

Phages of the χ (chi) type are characterized by a relatively small head and a thick tail equipped with a single long, wavy tail fiber. The original phage χ is specific to mobile bacteria of *E. coli*, *Salmonella* and *Serratia* (Meynell, 1961). The tail fiber is difficult to see and apparently easily removed by centrifugation. Despite their relatively thick tails, χ and its relatives are

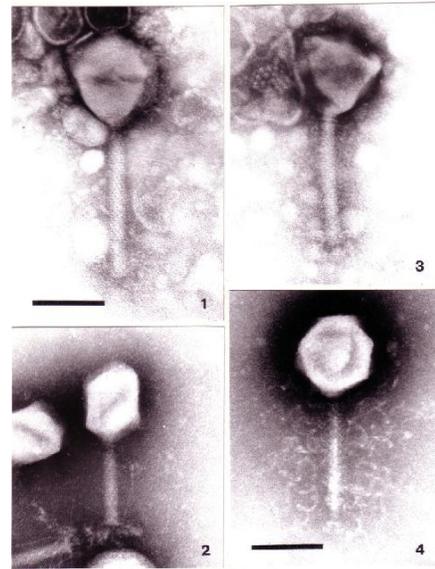


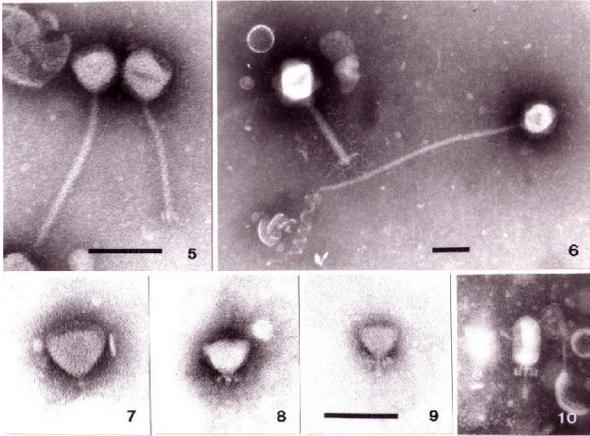
Figure 1-4. Myoviridae, morphotypes A41, T4, ϕ KZ, and LU11, respectively. Phosphotungstate, final magnification x 297,000; bars represent 100 nm.

siphoviruses. Type Jersey has a conspicuous base plate with six spikes. It was first found in *Salmonella paratyphi* B and seems to be restricted to *Salmonella* bacteria. However, the original phage Jersey has a tail of 116 nm in length only (Ackermann and Berthiaume, 1969) while the Jersey-like particles here have tails of much longer length (Figures 5 to 6). It may be speculated that they are tail length variants of the normal phage Jersey, phage Jersey in nature. Type T5 is indistinguishable from coliphage T5. Type S1 is morphologically unremarkable, but apparently has not been encountered elsewhere and may be novel. Type S2 is very interesting as it displays a single, rigid, thick, spiral tail fiber of approximately 200 nm in length.

The spirals have a periodicity of 47 nm. Prestel et al. (2008) observed similar particles in the sand of the Namibian desert.

Podoviridae

The N4 morphotype shows bundles of short tail fibers laying at the sides of the tail. Phages of this type seem to be very frequent in enterobacteria. Some of them are specific to capsulated bacteria, for example, *Klebsiella* and Vi antigen-containing *Salmonella* bacteria. However, the rather uncharacteristic morphology of these phages suggests that "type N4" is heterogeneous and comprises several genomically unrelated phages. The T7 type is very frequent in Gram-negative bacteria, especially enterics, pseudomonads and vibrios. It seems to be ubiquitous and to have a world-wide distribution. So far,



Figures 5-10: 5-6, Siphoviridae; 7-10, Podoviridae. 5, morphotypes χ (chi, left) and Jersey (right). 6, type Sx with long spiral tail fiber and an adventitious particle of type ϕ KZ. 7-10, Podoviridae. Types T7, N4, 44AJHD, and ϕ 29, respectively. Phosphotungstate. Final magnification x 148,500 in Figure. 6 and x 297,000 in all others; bars represent 100 nm.

all sequenced T7-like phages have shown genomic relationships (Lavigne et al., 2008). The types represented by *Bacillus* phage ϕ 29 and *Staphylococcus* phage 44AJHD occur in traces only. They differ in head shape (prolate in ϕ 29 and isometric in 44AJHD), but share genomic relationships (Lavigne et al., 2008) and a particular tail structure with a collar and collar appendages. The phages represent two different genera of the same phage subfamily, the *Nanovirinae* (Lavigne et al., 2008). Type ϕ 29 is ubiquitous and occurs not only in bacilli, but also in *Kurthia* and *Streptococcus* bacteria. Phages of types A41, ϕ KZ, Lu11, 121, T4, χ , Jersey, T7, ϕ 29 and 44AJHD are known to be virulent. This indicates that most phages here reported derive from lytic phage infections in the environment. At least eight types (ϕ KZ, 121, T4, χ , Jersey, N4, T7 and ϕ 29) seem to have a global distribution (Figures 7 to 10). This is consistent with metagenomic studies reporting the occurrence of T4 and T7 genes in the Arctic Ocean, the Gulf of Mexico and the Sargasso Sea (Angly et al., 2006) but remains to be ascertained by further ecological studies, especially in Third-World countries. It should be interesting to study the geographical distribution of phages and to investigate whether all phages of similar habitats occur world-wide or are limited to certain regions.

Electron microscopy for its rapidity and wealth of information it generates appears as the technique of choice for these investigations. We believe that we are just at the threshold of phage ecology and we have experienced that each ecological investigation of phage habitats in any country is likely to yield new and interesting phages.

REFERENCES

- Ackermann HW, Berthiaume L (1969). Ultrastructure des phages de lyso-typie des *Escherichia coli* 0127:B8. *Can. J. Microbiol.*, 15: 859-862.
- Ackermann HW, DuBow MS (1987). *Viruses of Prokaryotes. Vol II. Natural Groups of Bacteriophages.* CRC Press, Boca Raton, FL, pp. 72-121.
- Ackermann HW, DuBow MS, Gershman M, Karska-Wysocki B, Kasatiya SS, Loessner MJ, Mamet-Bratley MD, Regu e M (1997). Taxonomic changes in tailed phages of enterobacteria. *Arch. Virol.* 142:1381-1390.
- Ackermann HW, Krisch H (1997). A catalogue of T4-type bacteriophages. *Arch. Virol.* 142:2329-2345.
- Ackermann HW, Nguyen TM (1983). Sewage coliphages investigated by electron microscopy. *Appl. Environ. Microbiol.* 45:1049-1059.
- Angly FE, Felts B, Breitbart M, Salamon P, Edwards RA, Carlson C, Chan AM, Haynes M, Kelley S, Liu H, Mahaffy JM, Mueller JE, Nulton J, Olson R, Parsons R, Rayhawk S, Suttle CA, Rohwer F (2006). The marine viromes of four oceanic regions. *PLoS Biol.* 4:2121-2131 (e368).
- Bettarel Y, Bouvy M, Dumont C, Sime-Ngando T (2006). Virus-bacterium interactions in water and sediment of West African inland water system. *Appl. Environ. Microbiol.* 72:5274-5282.
- Grabow W, Holtzhausen C, de Villiers C (1993). Research on bacteriophages as indicators of water quality. WRC Report No. 321/1/93, Water Research Commission, Pretoria, South Africa.
- Hendrix RW, Casjens SR (2005). *Caudovirales*. In: Fauquet et al. (eds) *Virus Taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses*, Elsevier Academic Press. pp. 35-42.
- Kurtb ke DI, Murphy NE, Sivasithamparam K (1993a). Use of bacteriophage for the selective isolation of thermophilic actinomycetes from composted eucalyptus bark. *Can. J. Microbiol.* 39:46-51.
- Kurtb ke DI, Wilson CR, Sivasithamparam K (1993b). Occurrence of *Actinomadura* phage in organic mulches used for avocado plantations in Western Australia. *Can. J. Microbiol.* 39:389-394.
- Krylov VN, Dela Cruz DM, Hertveldt K, Ackermann HW (2007). " ϕ KZ-like viruses" - a proposed new genus of myovirus bacteriophages. *Arch. Virol.* 152:1955-1959.
- Lavigne R, Seto D, Mahadevan P, Ackermann HW, Kropinski AM (2008). Unifying classical and molecular taxonomic classification. Analysis of the Podoviridae using BLASTP-based tools. *Res. Microbiol.* 159:406-414.
- Lavigne R, Darius P, Summer EJ, Seto D, Mahadevan P, Nilsson AS, Ackermann HW, Kropinski AM (2009). Classification of Myoviridae bacteriophages using protein sequence similarity. *BCM Microbiol.* 9:224.
- Liu B, Wu SJ, Song Q, Zhang XB, Xie LH (2006). Two novel bacteriophages of thermophilic bacteria isolated from deep-sea hydrothermal fields. *Curr. Microbiol.* 53:163-166.
- Meynell EW (1961). A phage, χ , which attacks motile bacteria. *J. Gen. Microbiol.* 25:253-290.
- Nacesco N, Constantinesco SP, Petrovici A (1969). Aspects  lectrono-optiques du phage convertisseur *Proteus vulgaris* 121. *Arch. Roum. Pathol. Exp. Microbiol.* 28:838-848.
- Pasharawipas T, Thaikua S, Sriurairatana S, Ruangpan L, Direkbusarakum S, Manopvisetcharean J, Flegel TW (2005). Partial characterization of a novel bacteriophage of *Vibrio harveyi* isolated from shrimp culture ponds in Thailand. *Virus Res.* 114:63-69.
- Prestel E, Salamiou S, Dubow MS (2008). An examination of the bacteriophages and bacteria of the Namib desert. *J. Microbiol.* 46:364-372.
- Prigent M, Leroy M, Confalonieri F, Dutertre M, DuBow MS (2005). A diversity of bacteriophage forms and genomes can be isolated from the surface sands of the Sahara Desert. *Extremophiles* 9:289-296.
- Schaper M, Jofre J, Uys M, Grabow WOK (2002). Distribution of genotypes of F-specific RNA bacteriophages in human and non-human sources of faecal pollution in South Africa and Spain. *J. Appl. Microbiol.* 92:657-667.
- Stevenson RMW, Airdrie DW (1984) Isolation of *Yersinia ruckeri* bacteriophages. *Appl. Environ. Microbiol.* 47:1201-1205.
- Yu MX, Slater MR, Ackermann HW (2006). Isolation and characterization of *Thermus* bacteriophages. *Arch. Virol.* 151:663-679.