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Morphology of *Salmonella* Typhimurium typing phages of the Lilleengen set

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Abstract: The Lilleengen scheme for typing *Salmonella enterica* serovar Typhimurium consists of 12 tailed phages. Ten phages are podoviruses and morphologically identical to *Salmonella* phage P22. Two phages are siphoviruses and identical to flagella-specific phage χ.

Key words: morphology, phage typing, P22, *Salmonella*.

Résumé : Le schéma de Lilleengen pour la lysotypie de *Salmonella enterica* serovar Typhimurium comprend 12 phages caudés. Dix phages sont des podovirus et morpholgiquement identiques avec le phage P22 de *Salmonella*. Deux phages sont des siphovirus et de la même morphologie que le phage χ spécifique de bactéries flagellées.

Mots-clés : morphologie, lysotypie, P22, *Salmonella.*

[Traduit par la Rédaction]

Bacteriophage typing is an inexpensive, simple technique that has been enormously useful in epidemiology. The Lilleengen phage-typing scheme for *Salmonella enterica* serovar Typhimurium, published in 1948, was one of the first phage-typing schemes in use. The Lilleengen set comprises12 viruses isolated from sewage, manure, and Typhimurium cultures (Lilleengen 1948; Rabsch 2007). Their morphology has so far not been investigated. The typing set has been used successfully since 1961in combination with the Felix and Callow scheme by the Wernigerode Branch of the Robert Koch Institute (formerly Institute of Experimental Epidemiology) in Germany (Kuehn et al. 1973; Rabsch 2007).

The original bacteriophages and their propagating strain LT2 were obtained in 2004 from R. Wollin of the Swedish Institute for Infectious Diseases, Stockholm, Sweden. The phages were propagated in 20 mL of LB broth. After incubation for 4 h at 37 °C in a shaking water bath, lysates were freed from bacteria by repeated centrifugation at 3000*g* for 15 min and stored with a few drops of chloroform. Titers of 10^9–10^{10} were easily obtained. The propagation strain *S. enterica* serovar Typhimurium LT2 is devoid of Fels and Gifsy prophages. For electron microscopy, virus particles were sedimented at 25 000*g* for 60 min using a Beckman J2-21 centrifuge (Palo Alto, California) with a JA-18.1 fixed-angle rotor. Phages were then washed twice in 0.1 mol/L ammonium acetate (pH 7.0), deposited on copper grids provided with carbon-coated Formvar films, stained with 2% potassium phosphotungstate (pH 7.2), and examined in a Philips EM 300 electron microscope (Fig. 1). Magnification was monitored with catalase crystals (Worthington Biochemicals, Lakewood, New Jersey) (Luftig 1967).

Ten viruses, namely typing phages 2, 4, 8, 31, 32, 33, 34, 36, 37, and 39, showed isometric heads of 65 nm in diameter, 18 nm long tails, and conspicuous base plates with six spikes. They are members of the P22 genus of the Podoviridae family (Ackermann and DuBow 1987; Hendrix and Casjens 2005). Phages 28 and 33 were Siphoviridae with isometric heads of 64 nm in diameter and relatively long, thick, tapering tails of 225 nm × 10 nm with 57 or 58 cross-striations. Morphologically, they are members of the flagella-specific χ species of enteric phages (Ackermann and DuBow 1987; Meynell 1961), but we did not observe them adsorbed to bacterial flagella. The type virus of this species lyses *S. enterica* serovar Typhimurium, *Serratia marcescens*, and *Escherichia coli* (Meynell 1961) . The χ-like phages were observed independently in the laboratories of both H.-W. Ackermann and W. Rabsch.

The prevalence of P22-like viruses was expected. Several members of the Lilleengen scheme are known to be derived

Received 21 April 2009. Revision received 21 July 2009. Accepted 11 September 2009. Published on the NRC Research Press Web site at cjm.nrc.ca on 17 December 2009.

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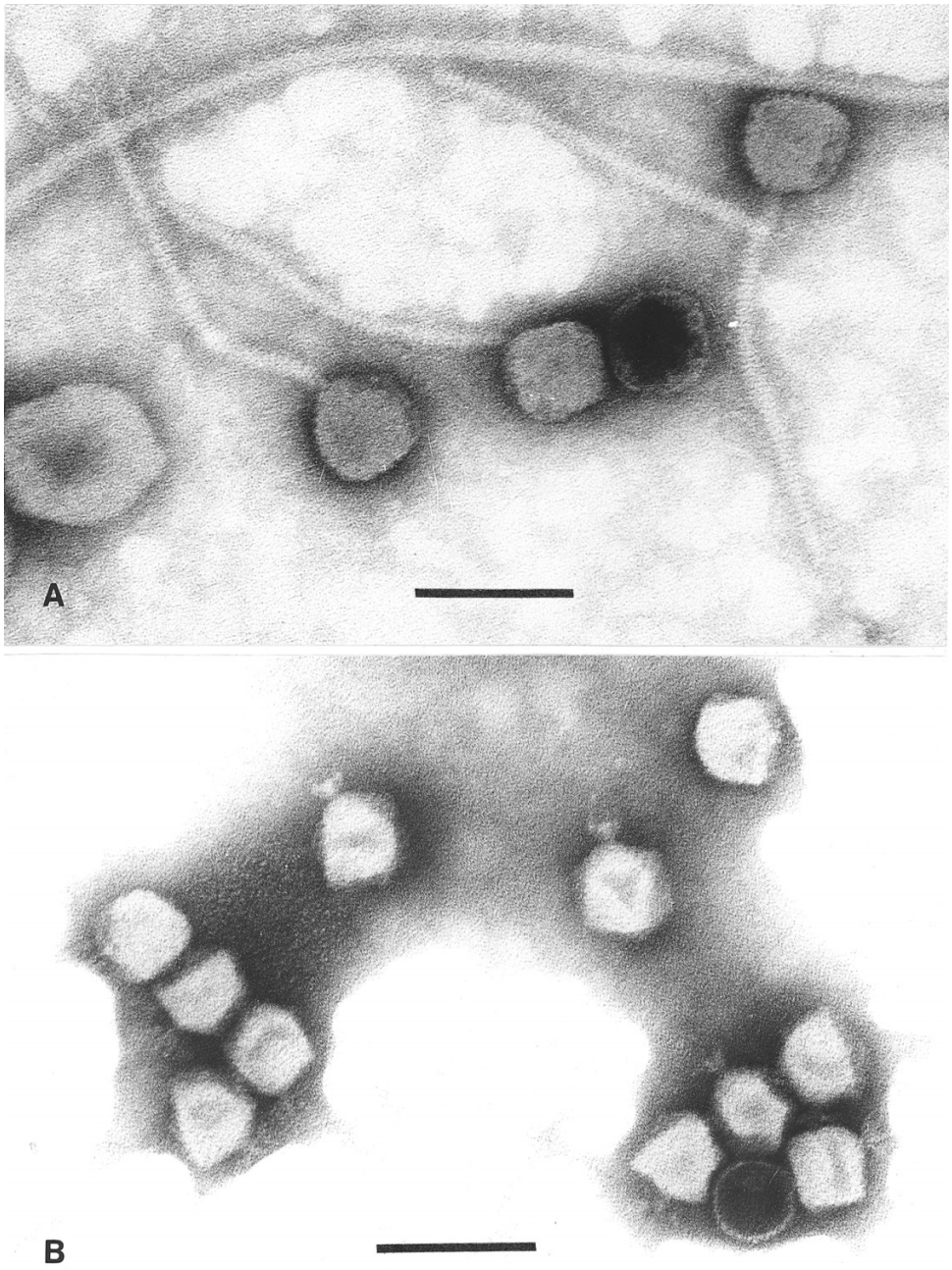
Fig. 1. (A) Phage 165 and (B) phage 33 stained with phosphotungstate. Final magnification is 297 000×; bars represent 100 nm.



from other typing phages by adaptation (Rabsch 2007). In addition, a previous investigation (Rabsch et al. 2007) of the phages of the Anderson *Salmonella* typing scheme had shown that all 31 members of this scheme are heteroimmune to P22, that all but two were related to P22 by DNA–DNA hybridization, and that most prophages harbored by propagating strains were of the P22 type (Rabsch et al. 2007; Schmieger 1999). In view of the temperate nature of phage P22, the P22-like particles in lysates of phage 33 appear to be adventitious. Their observation in the laboratories of H.-W. Ackermann and W. Rabsch indicates that they were present in the original lysates obtained from Stockholm. It is uncertain whether they interfere with lytic reactions in the typing scheme. It is clear that the identity of typing phages must be controlled by electron microscopy. The χ-like phages 28 and 33 have only superficial resemblance to phage H8 of the *Salmonella enterica* typing scheme of Lalko (1977), which has a much larger head and a shorter and thinner tailand closely resembles coliphage T5 (Rabsch et al. 2007).

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