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Isolation and characterization of a temperate cyanophage for a tropical *Anabaena* strain

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Abstract. In this paper we describe the isolation and characterization of a temperate cyanophage N(S)1 of the genus cyanopodovirus which produces turbid plaques on the host *Anabaena* 77S15 isolated from tropical soil. Its properties have been compared to those of other well-characterized cyanophages. In addition, two strains of *Anabaena* 77S15 lysogenic for N(S)1 were isolated. N(S)1 seems to be integrated into the chromosome of the two lysogens, and a 2 kb plasmid present at a low copy number in the non-lysogenic strain is amplified significantly.

Key words: Cyanophage – Cyanopodovirus – Cyano-

from the Pasteur Culture Collection (Rippka et al. 1979), Institut Pasteur, Paris, France. The tropical *Anabaena* and *Nostoc* (strains 74S08 to 79S03 and 74S04 to 79S04) were described by Franche and Reynaud (1986). The cyanophage N1 isolated by Adolph and Haselkorn (1971) was kindly provided by R. Rippka.

Cyanobacterial cultures

Cultures were maintained on slants of BG-11$_0$ medium (Rippka et al. 1979). For analysis samples were grown in 100 ml of BG-11 medium in 250 ml flasks incubated at 30°C

Table 1. Filamentous heterocystous cyanobacteria examined for susceptibility to cyanophage N(S)1

Genus	Strains	Reference
Anabaena	PCC7120, PCC7122 74S08[a], 74S12[a], 74S18[a], 74S19[a], 74S23[a], 74S24[a], 74S25[a], 74S26[a], 75S03[a], 75S19[a], *77S15*[a], 77S19[a], 79S01[a], 79S02[a], 79S03[a]	Rippka et al. 1979 Franche and Reynaud 1986
	Anabaena azollae var. *filiculoides*	Tel-Or et al. 1983
Nostoc	PCC73102, PCC 6720 74S04[a], 74S06[a], 74S07[a], 74S09[a], 74S22[a], 74S29[a], 74S51[a], 74S53[a], 74S54[a], 74S56[a], 74S60[a], 74S66[a], 77S17[a], 79S04[a], 79S05[a]	Rippka et al. 1979 Franche and Reynaud 1986
Scytonema	PCC7110	Rippka et al. 1979
Calothrix	PCC7102	Rippka et al. 1979
Fischerella	PCC7414	Rippka et al. 1979

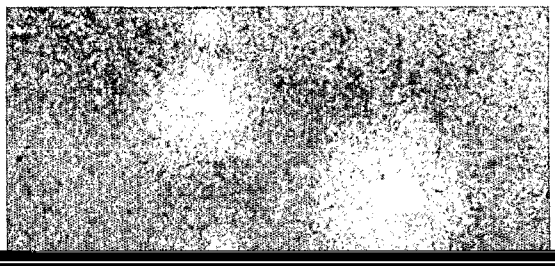
Electron microscopy of phage particles

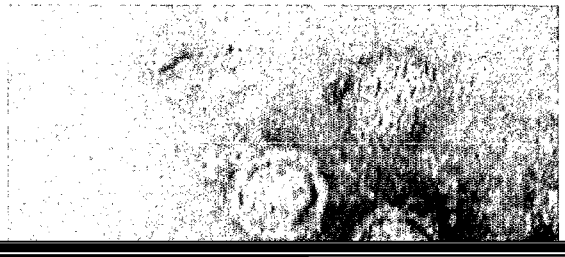
A phage stock was concentrated to $10^{11}-10^{12}$ particles per ml with polyethylene glycol 6000 (Elmerich et al. 1982). A drop of this suspension was applied to a 200-mesh copper grid coated with carbon and stained with 2% uranyl acetate. The grid was examined using a Siemens Elmiskop 101 electron microscope.

Adsorption and one-step growth experiments

medium for 3 weeks. The resulting cultures were then treated for 48 h with antiphage serum to precipitate free N(S)1 particles at a dilution of 1:10, washed three times, diluted 10-fold and cultured for 3 weeks in fresh BG-11 medium. Their supernatant were then checked for the presence of phage particles on overlays of the indicator strain *Anabaena* 77S15.

Mitomycin C treatment

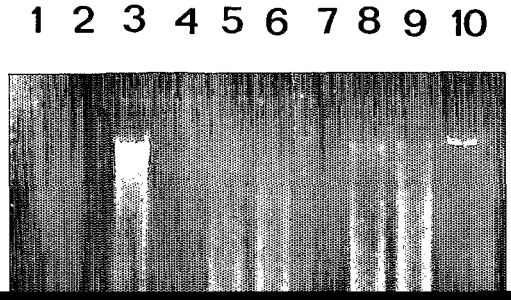




Phage N(S)1 was stable between pH 5 and pH 11. Loss of titer after 30 min of incubation at pH 4 or pH 12 were 40% and 35% respectively. Inactivation was complete after 5 min at pH 2 or pH 13 (data not shown).

Adsorption and one-step growth experiments

90% of the cyanophage were adsorbed on *Anabaena* strain 77S15 after 90 min (data not shown). Sodium citrate (10 mM) inhibited the attachment to the host-cell. The one-



tions were made: there is no homology between the 2 kb plasmid and DNA of the cyanophage N(S)1 (Fig. 4b, lane 10). On the contrary, both digested and non-digested DNA of *Anabaena* 77S15 showed significant hybridization with the plasmid of 84LS2 (Fig. 4b, lanes 7–9). Hybridization

duces clear plaques. We therefore attempted to isolate lysogens for N(S)1, and two stable lysogenic clones, 84SL1 and 84SL2, were obtained. The development of the prophage can be induced with mitomycin C as has previously been reported for the prophages of LPP-1D and LPP-2 of

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