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ISOLATION AND CHARACTERIZATION OF A PHAGE ACTIVE AGAINST STREPTOMYCES GRISEUS

By:

A.H. Esterabadi, F. Entessar, H. Hedayati, A.A. Narimani

A lytic agent isolated from the feces of a tuberculous calf was active only against Streptomyces griseus. Other types of this species and a number of Actinomyces strains were not susceptible. The phage after serial propagation by the agar plate technique, increased to 28 $\times$ 10 9 P.F.U per ml. The phage heat resistant at 60° C, was increasingly susceptible with increasing temperatures, only 5 P.F.U per ml was demonstrated after 15 minutes at 90°C. Electron microscopy showed that the phage had a hexagonal head with a long thin tail.

Bacterial phages are known for a variety of bacterial species. Reproduction of a bacterial phage on a sensitive host is in the most cases characterized by the lysis or disintegration of the cells of the host.

Saudek and Colingworth (8) reported a bacteriophage that attacked Streptomyces griseus. The lytic activity of the phage was demonstrated on solid media, by the formation of plaques, and in liquid media, by partial prevention of streptomycin production. Subsequently Woodruff et al (9) isolated a phage active against Streptomyces culture. He showed that the virus is particulate and transmissible producing lysis in young cells of Streptomyces griseus.

Phages capable of infecting various Streptomyces species have been reported by Reilly et al (7) Koerber et al (5) perlman et al (6) Alexander and McCoy (2) and Gilmour et al (4).

This paper reports the isolation and charaterization of S. griseus phage, which was recovered from intestinal contents of a calf suffering from tuberculosis.

MATERIALS AND METHODS

Media

Nutrient agar contained 1% Bacto peptone, 0. 5% meat extract, 0.5%

sodium chloride and 2% agar for solid medium, and 0.7% agar for overlayer culturing of the phage at pH7.2, both media contained the same ingredients except agar.

Isolation and propagation of the phage.

Moth-eaten like colonies were observed on a nutrient agar culture the feces of a calf suffering from tuberculosis. The lytic agent was isolated from a suspension of the colonies by filtration through a Millipore type H.A filter, pore size 0.45μ The isolated phage was propagated by the double agar layer technique as described by Adams (1).

Streptomyces culture was suspended in 1 ml of nutrient broth and mixed with 1 ml of the phage containing filtrate. The mixture was added to an equal volume of the 0.7% agar medium at 45° C, immediately poured over nutrint agar plate and uniformly spread with a glass rod. The plates were incubated for 48 hours at 37° C. The phage was harvested in peptone solution and held at 4°C for 48 hours. The agar and cells were removed by filter paper then, the filtrate was passed through a millipore filter pore size 0.45μ This procedure was repeated until maximum concentration of plaque forming unit (P.F.U) was demonstrated by titration.

Phage activity

Gross activity was determined by adding a mixture of I ml of the phage filtrate and about 10^9 young host organism in 1 ml peptone solution to an agar plate, incubating for 48 hours.

Titration of the phage was made by placing a drop of 10 fold serial dilutions in peptone broth on plate seeded with host organism respectively. The plates were incubated for 48 hours. Other strains from different sources of Streptomyces species as well as Actinomyces strains were tested by placing drops of the phage suspension on agar plates seeded with the organisms.

Heat susceptibility

Aliquots of the phage suspension containing 28×10^9 P.F.U were held in a water bath for 15 minutes at temperatures ranging from 60° C to 90° C. Susceptibility was determined by titration of the P.F.U.

Electron microscopy

The phage for photography was prepared by using 1 ml of phage suspension removed from plate that showed complete when plated with the S. griseus.

After incubation, the plates showed many plaques, the phages were extracted with 0.01 M ammonium acetate solution PH 7.2.

The mixture was held at 4°C for 24 hours, then filtered through a 0.45μ Millipore filtrer. The filtrate was centrifuged at 30,000 r.p.m for 2 hours.

The sediment containing the phage was suspended in the ammonium acetate solution. The suspension was clarified by low centrifugation at 6,000 r.p.m for 30 minutes. The supernatant was centrifuged at 30,000 r.p.m for one hour.

The phage in the sediment was sufficiently clear for the electron microscopy. The negative staining method described by Brenner and Horne (3) was used. Dilutions of the phage suspensions were mixed with equal wolume of neutral 2% phosphotungstic acid. The pH was adjusted to 7.0 with potassium hydroxide. The preparations were placed on Formvar covered electron microscope girds and examined with a Siemens Elmiscop I operated at 60 KV.

RESULTS

The smooth opaque colonies of the S. griseus was shown in Fig 1. The morphology of the mycelium of the respective colonies are shown in Fig 2 and 3. Typical plaques are shown in Fig 4.

The phage when freshly isolated had a titer of 10^6 P.F.U. per ml. After four consecutive passages the titer increased to 28×10^9 P.F.U. per ml.

The phage was highly specific, with activity only on the host strain of S. griseus which was isolated. By increasing the phage concentration, no changes were observed on cultures of different species of streptomyces and Actinomyces were tested for lysis by the phage at RTD and 10,000 RTD. (Table I).

The phage was resistant to 60° C for 15 minutes and that at incraseingly higher tempratures the number of P.F.U generally decreased at 85° C. There was a precipitous drop at 90° C, there were only 5 P.F.U (Table II).

The phage particle head was hexagonal (700 A° long and 600 A° wide) and to which was attached a thin tail (2400 A° long and 6 A° wide) that appeared to be straight or slightly curved, usually terminated with a knob. (Fig 5,6).

DISCUSSION

In the present study a phage isolated from the intestinal contents of a tuberculous calf was found to be specific for Streptomyces isolated. Other strains

of Streptomyces and Actinomyces obtaind from different sources were not susceptible to this phage.

The isolation of Streptomyces griseus phage from various sources has been reported by Saudek and Colingsworth 1947, Woodruff et al 1947, Gilmour et al 1956, and Badley and Kay 1960. Morphologically, as determined by electron microscopy, the present phage was similar to those previously isolated except that it was larger in size. The terminal knob had cross striation along the axis and no collar was obsdrved between the head and tail.

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Table: I

Results of comparison of phage activity on different of Streptomyces and Actinomyces strains

Description of Strain	oringin of culture	Susceptibility to phage	
		RTD	10; 000 RTD
Streptomyces grieus	Iran	CL	CL
» somaliensis S.N°35	U.S.A	NL	NL
» pelletierii S.N°36	U.S.A	NL	NL
» madurae S.N°39	L.S.H.T.M	NL	NL
» pargoyensis S.N°56	Iran	NL	NL
Actinomyces asteroides S.N°33	Sudan	NL	NL
» » brasiliansis S.N°31	»	NL	NL
» » caviae S.N°32	»	NL	NL

CL = Confluent lysis
NL = NO lysis
L.S.H.T.M = London School of Hygien Tropical Medicine

Table: II

Effect of temperature upon the stability of phage (At start, 28×10^9 P.F.U per ml)

Temperature C°	Phage particles perml, after 15 minutes
Control (37°C)	28×10^9
60	28×10^9
65	6×10^9
70	2×10^9
75	1×10^9
80	2×10^7
85	20
90	5

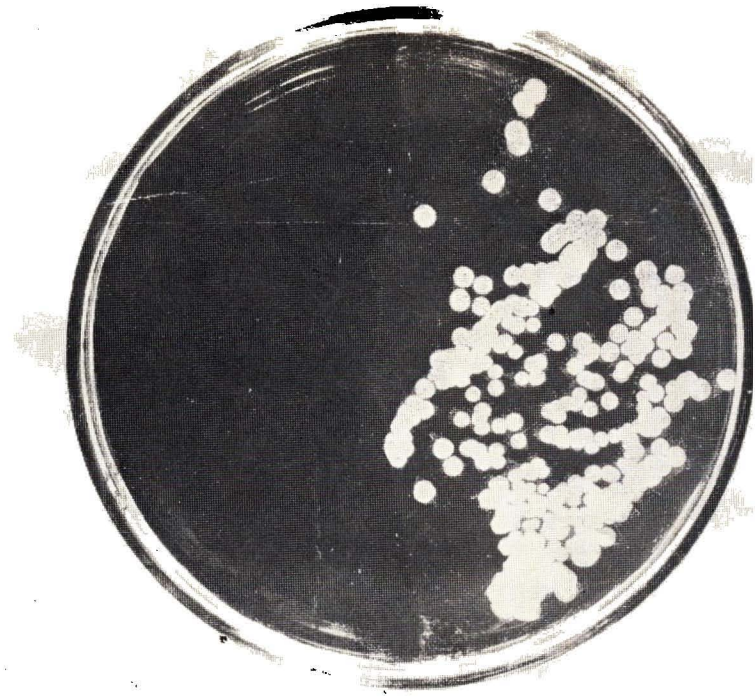
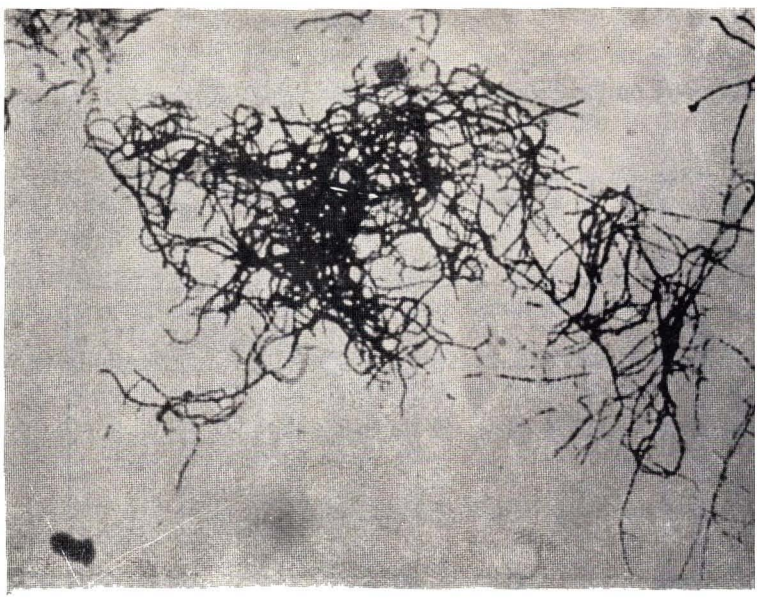


Fig: 1- Normal colony of S. griseus



Fiz: 2- Normal mycelium

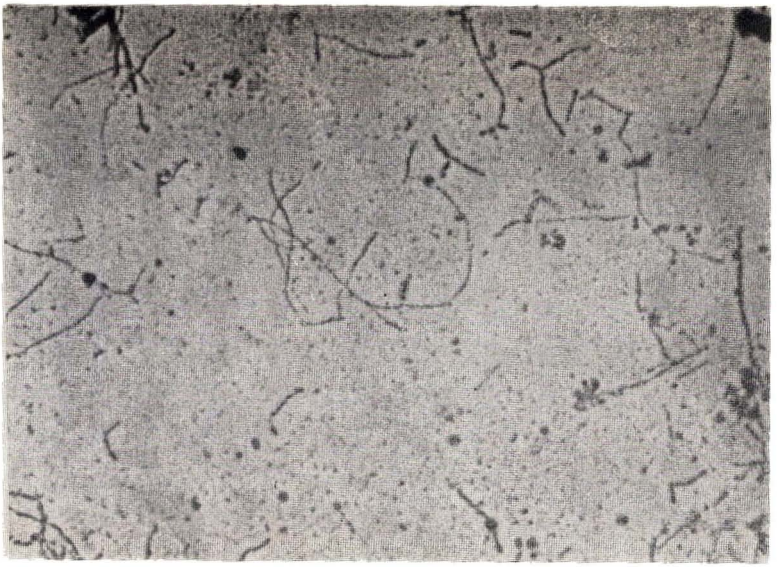


Fig: 3- Phage infected mycelium

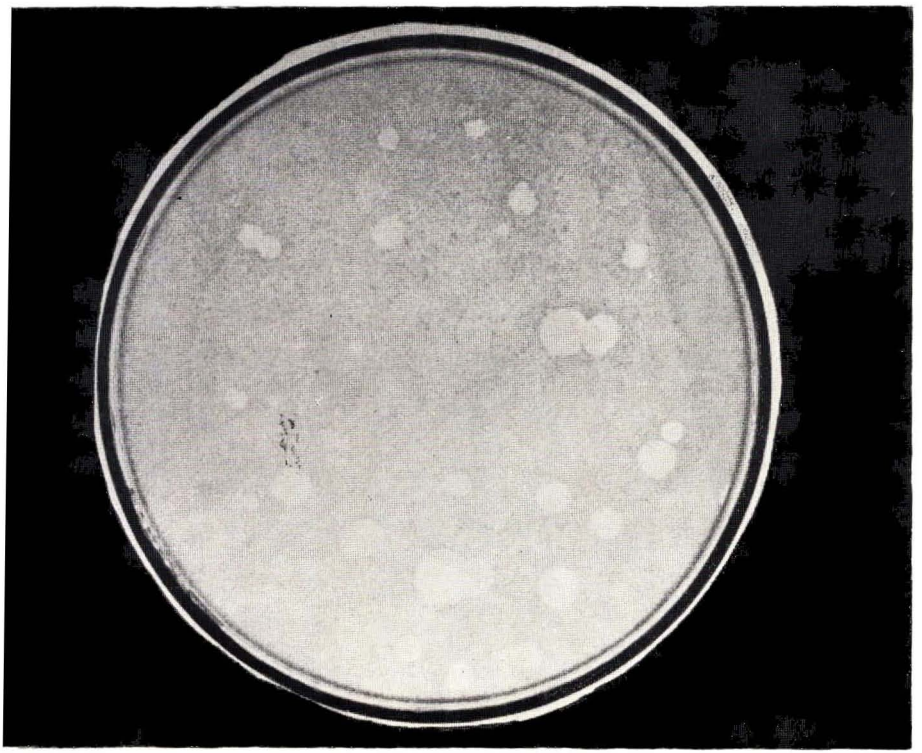


Fig: 4- Phage plapues

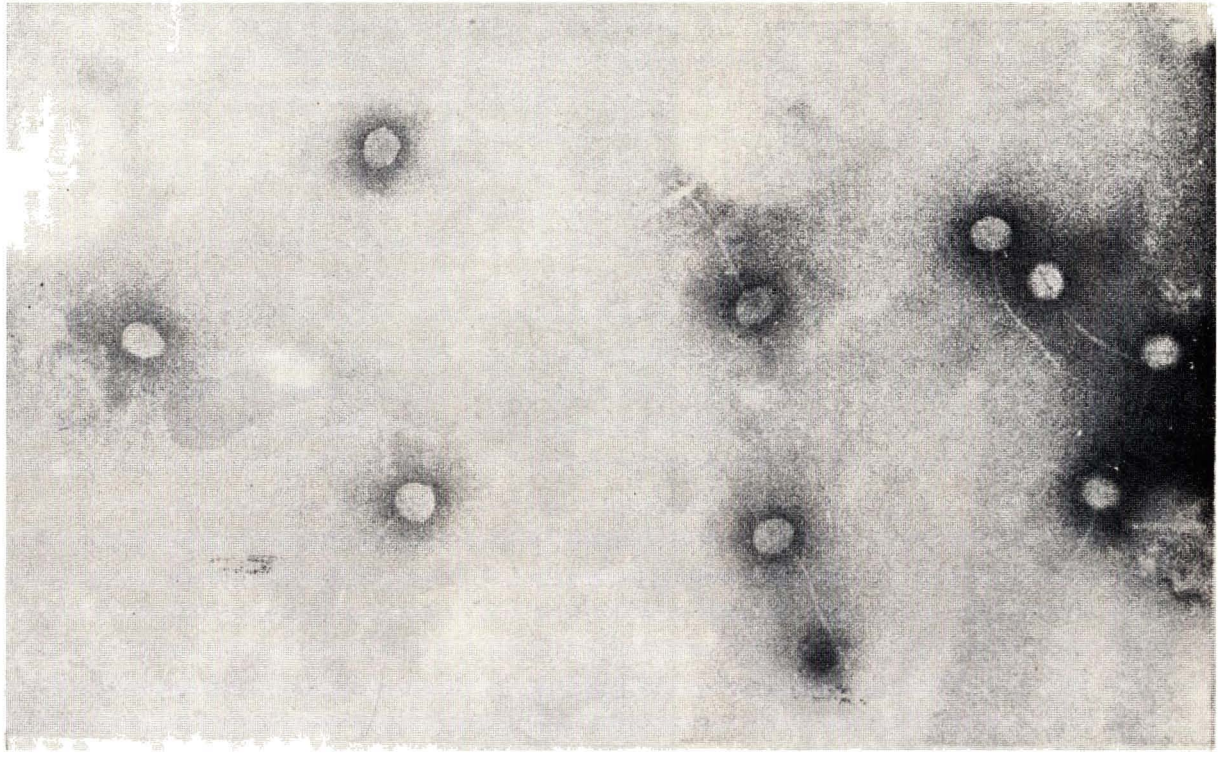


Fig: 5,6- Electron micrographs of S. griseus phage PTA (X 80,000)