

of interest to find that the patterns of infectiousness are different for the embryo and for the fully developed structures. In the embryo, the brain tissues seem to be particularly susceptible to Influenza-A virus, whereas in the adult the respiratory mucous membranes are primarily affected. In mumps, the infection of the salivary glands is not in-

frequently combined with meningitis, but no effect on the brain was found macroscopically in embryos. The situation is the same as in rubella where the embryonic defects seem to have no obvious relations to the manifestations of rubella infections in older phases of life.

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A Rapid Method for Demonstrating the Identity of Streptomycin-Producing Strains of *Streptomyces griseus*.^{*†}

SELMAN A. WAKSMAN, H. CHRISTINE REILLY, AND DALE A. HARRIS.

From the New Jersey Agricultural Experiment Station, New Brunswick, N. J.

In a search for new strains of the streptomycin-producing organism, *Streptomyces griseus*, it is usually necessary to make a large number of isolations from the soil or other natural substrates, test the ability of the organisms to inhibit the growth of various bacteria, grow them in liquid media favorable for the production of the antibiotic, isolate the latter from the medium, and establish its identity with streptomycin. Out of a hundred or more cultures of *S. griseus* thus isolated and tested,^{1,2} only very few were found capable of producing streptomycin. Most of the cultures formed no antibiotic at all, whereas some produced other antibiotics, such as grisein.³

To overcome this difficulty, two procedures were adopted: 1. The use, for isolation purposes, of media containing streptomycin, so as to eliminate most of the bacteria and the great majority of actinomycetes that are sensitive to streptomycin. 2. The use of *S. griseus*

actinophage for rapid spotting of the streptomycin-producing strains of *S. griseus*, since these were found⁴ to be sensitive to this actinophage, whereas all other strains of *S. griseus* were resistant.

The results of the following experiment illustrate the use of these procedures. One gram samples of soil, cow manure, and compost materials were plated out, in dilutions of 1:10⁴, 1:10⁵, 1:10⁶, and 1:10⁷, with nutrient agar containing 25 µg of streptomycin per ml. The plates were incubated at 28°C for 5 days. Five actinomycetes colonies were isolated from several of the plates and grown upon slants of glucose-asparagine agar. Good growth was obtained after 7 days' incubation.

The 5 cultures were streaked on nutrient agar plates and incubated for 24 hours; drops of actinophage, both undiluted and diluted 1:10, were placed upon some of the streaks. The plates were again incubated for 24 hours at 28°C, and examined; the streaks that were treated with actinophage showed inhibition of sporulation and lysis of the vegetative mycelium.

A duplicate set of plates was streaked with the freshly isolated cultures, incubated for 48 hours, and cross-streaked with four test bacteria.⁵ The plates were incubated for an additional 48 hours, and the zones of bacterial

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⁴ Reilly, H. C., Harris, D. A., and Waksman, S. A., *J. Bact.*, 1947, **54**

TABLE I.
Zones of Inhibition of Test Bacteria by Freshly Isolated Strains of *S. griseus*, as Measured by
Agar-streak Method.

Zone of inhibition measured in millimeters.

Strain No.	<i>Escherichia coli</i>	<i>Bacillus mycoides</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
1	20	20	18	26
2	23	23	19	28
3	20	20	17	27
4	22	22	20	26
5	20	22	17	23

TABLE II.
Production of Streptomycin by Freshly Isolated
Strains and Stock Culture of *S. griseus*.
Streptomycin, $\mu\text{g/ml}$.

Strain No.	Days of incubation		
	3	5	7
1	30	120	93
2	42	63	60
3	55	132	128
4	40	54	54
5	43	108	96
3475	42	81	65

inhibition recorded. The results (Table I) show that all the cultures were able to inhibit the growth of both gram-positive and gram-negative bacteria, the type of spectra obtained being reminiscent of streptomycin.⁶

The 5 cultures were inoculated into flasks with meat extract-peptone-glucose medium commonly used for the production of streptomycin. For comparative purposes, an active streptomycin-producing strain of *S. griseus* (No. 3475) was also used. The flasks were

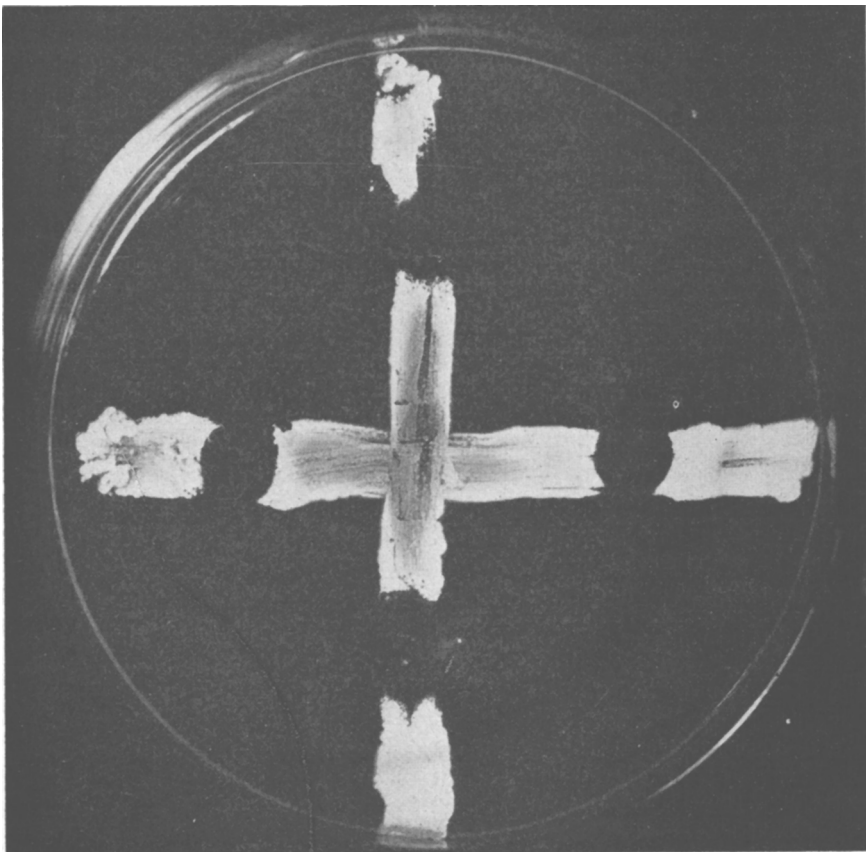


FIG. 1.
Use of actinophage for identifying streptomycin-producing *S. griseus* cultures.

placed in a shaking machine in the incubator at 28°C. The activity of the culture filtrates was determined after 3, 5 and 7 days incubation by the cup method against a streptomycin standard (Table II).

The results show that the freshly isolated cultures were all active producers of streptomycin. That the antibiotic formed by the unknown strains of *S. griseus* was streptomycin or at least a streptomycin-like substance is illustrated by the similarity in the antibiotic spectra of the different preparations.

To illustrate further the effect of phage upon the unknown strains, the cultures were streaked upon agar plates. These were incubated for 24 hours, metal rings placed upon a portion of the area of growth of the organisms, and a few drops of actinophage placed inside each ring. The plates were incubated another 24 hours, the rings removed, and the plates examined. The action of the phage is illustrated in Fig. 1. The effect of the phage

was exactly the same as that usually produced on the streptomycin-producing strains of *S. griseus*.

Summary. A method for the rapid isolation and identification of streptomycin-producing strains of *Streptomyces griseus* is described. The method consists in plating out various dilutions of material from natural substrates, using agar media to which 25 µg/ml streptomycin has been added. Colonies of actinomycetes developing on the plates are picked and transferred to agar slants. When the cultures have developed, they are used to make agar streaks on plates. The plates are incubated for 24 hours, and drops of actinophage placed upon some of the streaks. The destruction of the growth of the actinomycetes will prove the identity of the culture with streptomycin-producing *S. griseus*. This identification can be confirmed by the use of the agar-streak method, using various test bacteria, the sensitivity of which to streptomycin is known. Finally the cultures are grown in media used for the production of streptomycin, and the antibiotic produced is compared with streptomycin.

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Enhancement of Heterophile and Bacterial Agglutination Titers by Means of Serum Diluent.*

ALBERT MILZER AND SHIRLEY NATHAN.

From the Department of Bacteriology, Research Institute, Michael Reese Hospital, Chicago, Ill.

Various workers have shown that Rh agglutination titers are enhanced, and the action of the so called "blocking" antibody inhibited by the use of serum, plasma or bovine al-

bumin instead of saline as a diluent.¹⁻⁶ Recently Griffiths⁷ has reported the inhibition of Brucella blocking antibodies when rabbit serum is used in place of saline as a diluent in agglutination tests. This paper is a preliminary report on the enhancement of heterophile and various bacterial agglutinin titers by use of serum diluent instead of physiological saline. Routine agglutination tests were done comparing saline and pooled human

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