Table 1. Host ranges of streptomyces-phages and nocardia-phages with respect to several genera of the Order Actinomycetales. SP- and NP- denote phages initially isolated on streptomycetes and nocardia respectively. Lysis of a host by a standard phage suspension is indicated by a plus sign; no lysis is denoted by a minus sign.

Hosts	Actinophages					
	SP-3	SP-4	SP-8	NP-3	NP-4	NP-5
Streptomyces griseus S34	−	+	−	+	−	−
Streptomyces griseus S104	+	+	+	+	+	+
Streptomyces griseus 1945	−	−	+	+	+	−
Streptomyces olivaceus S11	+	+	+	+	+	−
Streptomyces venezuelae S13	+	+	+	+	+	+
Streptomyces cyaneus S45	−	−	−	+	−	−
Nocardia sp. 3403	−	−	−	−	−	+
Nocardia paraguayensis	+	+	+	+	−	−
Nocardia madurae	−	−	−	−	+	−
Nocardia brasiliensis	−	−	−	−	−	−
Nocardia asteroides	−	−	−	−	−	−
Actinoplanes sp.	−	−	−	−	−	−
Streptosporangium sp.	−	−	−	−	−	−
Mycobacterium phlei	−	−	−	−	−	−
Micromonospora sp.	−	−	−	−	−	−

monospora and *Mycobacterium* were from the culture collection maintained at the University of Minnesota. The general precedures for isolating and purifying actinophages followed those previously published (*6*). The nutritive substrate used in earlier work did not support rapid growth of all the organisms studied here. Therefore a modified medium was devised, containing the following ingredients: Difco peptone, 5 g; Difco yeast extract, 3 g; glucose, 1 g; Difco casamino acids, 1 g; Armour beef extract, 2 g; Difco agar, 15 g; deionized water, 1 liter.

Eleven soil samples, collected in Minnesota, have yielded three streptomyces phages and five nocardia phages. Of the three newly isolated streptomyces phages, one attacked *Nocardia paraguayensis* as well as several members of the genus *Streptomyces* (Table 1). Of the five newly isolated nocardia phages, three attacked streptomycetes. The strains of *Actinoplanes, Streptosporangium, Micromonospora,* and *Mycobacterium* used in this investigation were resistant to all streptomyces phages and nocardia phages tested.

At the present time, the genera *Actinomyces* and *Nocardia* are taxonomically placed in the family Actinomycetaceae, whereas *Micromonospora* and *Streptomyces* are placed in the family Streptomycetaceae. In view of the results reported here, it seems that the genera *Nocardia* and *Streptomyces* are closely related and should not be separated into different families. This interpretation is supported by the recent discovery that *Nocardia asteroides* produces conidiospore-like structures (*7*). The formation of chains of conidiospores is one of the fundamental criteria for differentiating *Nocardia* and *Streptomyces*. In fact, it has been recently pointed out that there is no clear-cut distinction between the genera *Nocardia* and *Streptomyces* and that the separation of *Streptomyces* from *Nocardia* is invalid (*8*).

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Detection of Ultraviolet Absorbing Spots on Paper Chromatograms by Blueprint Paper

Many organic compounds having strong ultraviolet absorption at 250 to 260 mμ can be detected on paper chromatograms by the photographic method of Markham and Smith (*1*) or Haines and Drake (*2*). The chromatogram is used as a "negative" over a sheet of contact printing paper. The light source is a quartz mercury arc, used with a filter that transmits primarily the intense 253.7-mμ line. Substances having high extinction at this wavelength show as white spots on a black background when the print is developed. Several refinements of this technique have been proposed (*3, 4*), but it requires a darkroom and the usual developing and fixing baths which are not conveniently available in many laboratories.

This report (*5*) describes the use of ferric ferricyanide blueprint paper, which is relatively insensitive to visible light and can be handled without special precautions in any well-lighted laboratory (though direct sunlight must be avoided). It can be developed in a few seconds by rinsing with cold tap water. Excellent "prints" are obtained even with an inexpensive unfiltered mercury arc light. The cost of blueprint paper is less than one-fifth that of photographic printing paper.

Blueprint paper is available from several manufacturers and in various grades. All will probably give satisfactory results, although there will be minor differences in speed, depth of color, and contrast. In this work, Dietzgen XL, a thinly-coated paper, has been used. It seems to give somewhat greater exposure latitude, lighter blue background, and better-defined spots than some other papers.

Two kinds of low-wattage ultraviolet lamps are useful. One is a quartz "germicidal" mercury arc emitting over 90 percent of its energy at 254 mμ. The other is a "black light" fluorescent lamp, whose phosphor emits most of its energy in the long-wave ultraviolet near 360 mμ. Both lamps also emit visible light, but filtering is unnecessary because of the low sensitivity of blueprint paper to wavelengths above 400 mμ. The lamps are mounted on a frame about 2 inches above the paper. For one-dimensional chromatograms up to 2 inches wide and 12 inches long, one lamp is sufficient; for wider chromatograms several parallel lamps must be used (or a single high-wattage lamp set 12 inches or higher above the paper). Complete equipment and materials needed for ultraviolet blueprinting of chromatograms are obtainable from Microchemical Specialties Company, Berkeley, Calif.

The technique has been used so far primarily with test strips and one-dimensional chromatograms of Whatman No. 4 paper (available in 600-foot rolls and widths of ½, 1, 1½, . . . inches). Blueprint paper is taken from a 2-inch wide roll in a dispenser and laid on a thin, flexible-plastic, 12-inch rule. The filter paper strip is laid on the sensitized surface of the blueprint paper, and paper clips are used to attach the ends of the papers to the plastic rule. If better contact is desired, more clips may be used at intervals of 2 inches; the clips show

on the print, but this does not interfere with detection of spots. For two-dimensional chromatograms, a printing frame is desirable (*4*). Poor contact gives less well-defined spots and slightly less sensitive detection.

Exposure time is much longer than for photocopy paper and is not very critical, but a photographic printing timer is useful. Correct exposure should be determined for the particular lamp, filter paper, and blueprint paper used. With Whatman No. 4 paper, at 254 mμ, a 1-minute exposure gives a very pale blue background, and spots are detected with maximum sensitivity. A 3-minute exposure gives a darker background, against which spots show with maximum contrast and definition but with slightly less sensitivity. Longer exposures simply lower the sensitivity of detection and are useful only if it is desired that weak spots not show on the print. At 360 mμ, exposure time is about half that at 254 mμ. With thick papers a 10-minute exposure may be necessary, unless a solution of 25 percent heavy white mineral oil in isooctane is sprayed or poured on the paper. The increased transparency reduces exposure time by a factor of 2 to 3, and improves the definition of spots. It lowers sensitivity of detection, however, by a factor of 2 to 3, because the intensification ("hyperchromic absorption") of spots on dry filter paper is lost (*6*).

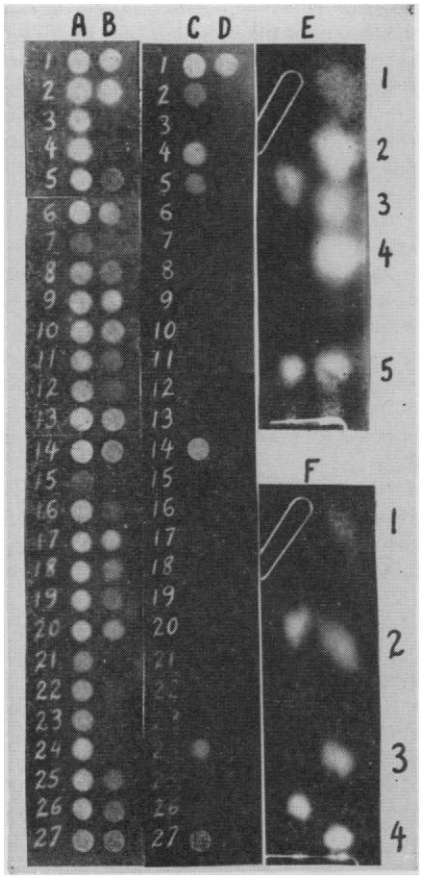

Fig. 1. Blueprints of ultraviolet spots on Whatman No. 4 filter paper. Columns A to D are 2-μl test spots. Solutions in columns A and C are 0.05*M* (0.1 μmole), in columns B and D, 0.005*M*. (0.01 μmole). A and B blueprinted at 254 mμ, 1 minute, C and D at 360 mμ, 1 minute. Identification: 1, pyrene; 2, phenanthrene; 3 acenaphthene; 4, acenaphthylene; 5, ergosterol; 6, testosterone; 7, estradiol; 8, DDT; 9, DDE; 10, sulphenone; 11, sevin; 12, kelthane; 13, mitox; 14, rotenone; 15, 2,4-D; 16, methadone; 17, adenosine; 18, nicotinamide; 19, pyridoxine; 20, ascorbic acid; 21, tryptophan; 22, maleic acid; 23, fumaric acid; 24, potassium ferricyanide; 25, sodium molybdate; 26, copper acetate; 27, ferric chloride. Columns E and F are chromatograms, with β-methoxypropionitrile as the stationary phase and isooctane as the mobile phase, blueprinted at 254 mμ, 3 minutes. The spots on the left are azo dyes used as reference standards. Spots on right are: Chromatogram E (all 0.08 μmole): 1, fluorene; 2, methyl cholanthrene; 3, 7,8-benzoquinoline; 4, 3,4-benzoquinoline; 5, acenaphthenol. Chromatogram F (all 0.1 μmole): 1, diazinon; 2, trithion; 3, parathion; 4, guthion.

After exposure, the blueprint paper is washed in cold water under the tap for 5 to 10 seconds. The paper is then pressed flat on a ferrotype plate or other smooth flat surface and allowed to dry for a few minutes. To prevent curling the paper must be removed before it is completely dried and pressed flat between sheets of blotter paper for 10 minutes or more. Starting lines, solvent front lines, and other notations in ink or pencil on the chromatogram are blueprinted like the ultraviolet spots, so that R_f can be measured directly on the print.

If a chromatogram or spot test is to be blueprinted both at 254 and at 360 mμ, the test at 360 mμ must be done first, because the exposure at 254 mμ may cause chemical changes in the absorbing substance that cause the spot to absorb at 360 mμ or even to develop a visible color. Pyridine derivatives, such as nicotinamide, for example, are not detected by blueprinting at 360 mμ, but the decomposition products formed during blueprinting at 254 mμ cause the spot to turn yellow and to absorb strongly at 360 mμ.

Ultraviolet-sensitive papers other than ferric ferricyanide blueprint paper have been tried and found to be less satisfactory. Diazotype papers give dark spots on a white background, but neither the sensitivity nor the contrast is as good as that of blueprint paper. Ferric-silver "Vandyke" papers (such as Dietzgen No. 227) give white spots on a dark brown background but are more expensive and not significantly better than blueprint paper.

By using an intense high-pressure mercury arc and a nickel sulfate–cobalt sulfate solution in a Lucite cell as a filter, Bernasconi *et al.* (*7*) obtained a narrow ultraviolet band (300 to 350 mμ) useful for selective photographic detection of compounds with maxima in this region. The filter system, however, would probably transmit too little light to be suitable for the relatively insensitive blueprinting method, although it is satisfactory with silver halide papers.

Sample blueprints of spot test strips and of chromatograms are shown in Fig. 1. If the molecular extinction coefficient of a substance at 254 mμ is of the order of 10,000, a spot of 0.001*M* solution can be detected. Absorption at 254 mμ has been used for detecting purines and pyrimidines (*1*) and ketosteroids such as cortisone (*3, 8*). Figure 1 shows that blueprint paper is also useful for detecting certain sterols, insecticides, acaricides, many aromatic compounds, and some inorganic ions. Absorption at 360 mμ is of more limited use but can be used to detect polynuclear aromatics and other compounds with long sequences of conjugated double bonds, some aromatic nitro compounds, and a few inorganic ions.

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Determination of Deuterium Oxide in Water by Measurement of Freezing Point

A simple, rapid method for determination of deuterium oxide (D_2O) in water has been devised. The most widely used and precise methods involve use of the mass spectrometer and the falling drop. Other less common techniques include measurements of density gradient, phase-contrast refraction, sinking rate of a quartz float, infrared absorption, and thermal conductivity. No reference has been found to a method based on elevation of the freezing point of water.

The freezing point of deuterium oxide