APPLIED MICROBIOLOGY, July 1967, p. 723-725

Vol. 15, No. 4
Printed in U.S.A.

Application of Paper Chromatograms to the Study of Inducers of λ Bacteriophage in *Escherichia coli*

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Received for publication 16 December 1966

A procedure has been developed whereby paper chromatograms of agents which induce λ bacteriophage in *Escherichia coli* can be developed using bioautographs with a lysogenic test system. Well-defined plaque-forming zones are produced indicating the area on the paper chromatogram where the active inducing material can be located. A mixture of the bacteriophage-inducing antibiotic, mitomycin C, and the noninducing antibiotic, paromomycin, was resolved into its components on paper strips with an ethyl acetate-methanol solvent system. The location of both antibiotics could thus be readily observed. Antibacterial and inducing activities were found to be identical with a crude fermentation solid, NSC-B-158,791. The use of this procedure for resolution of multicomponent inducing activities in antibiotic beers and for characterization of active components which may be potential antitumor antibiotics is indicated.

Reports from several laboratories (1–10) suggest the utility of a test system employing induction of bacteriophage in lysogenic bacteria as an assay tool for detection and isolation of antineoplastic agents. In our laboratory, development of a simple, rapid, quantitative assay (4, 8) which measures induction of λ bacteriophage in lysogenic *Escherichia coli* has greatly stimulated our screening of fermentation beers for antineoplastic antibiotics. Approximately 25 purified antibiotics which have a significant induction index and also display antitumor activity in rodent systems have already been described.

The need for paper chromatographic analysis for resolution of multicomponent bacteriophage-inducing activities in antibiotic beers and for the characterization of active components during fractionation and purification, as well as for the recognition of identical inducing factors in different antibiotic beers, soon became apparent.

This report describes the use of a lysogenic system for locating the position of bacteriophage-inducing antibiotics chromatographed on paper strips.

MATERIALS AND METHODS

Microorganisms. The lysogenic bacterium *E. coli* K-12 (λ) and the indicator culture *E. coli* C600 were maintained separately on nutrient agar (Difco Nutrient Broth, dehydrated, 0.8% and Difco agar, 1.8%) slants.

Culture preparation. A suspension (optical density of 0.2, determined with a Bausch & Lomb Spectronic-20 colorimeter at 530 mμ) of *E. coli* K-12 (λ) in sterile saline was prepared from a slant. The suspension was washed twice and diluted 1:2,000 in sterile saline; 1 ml of the diluted suspension was added to 100 ml of melted soft agar (Difco Nutrient Broth, dehydrated 0.8%; NaCl, 0.5%; and Difco agar, 0.5%). *E. coli* C600 was grown in nutrient broth (Difco Nutrient Broth, dehydrated, 0.8%; and NaCl, 0.5%) for 18 hr at 37 C and diluted 1:10 with saline; 2.5 ml of the saline suspension was then added to 100 ml of the melted soft agar along with the lysogenic bacterium.

Preparation of agar overlay. Sufficient MacConkey Agar (BBL) was poured into the bottom of a dish to form a base layer. After the base layer hardened, a thin layer of inoculated soft agar was poured on the surface of the base layer and allowed to harden.

Paper chromatography. Strips (0.5 inch) of no. 589 blue ribbon paper (Schleicher & Schuell Co., Keene, N.H.) were used. The test materials were applied at the 1-inch point. Chromatograms were developed by downward movement in chromatography chambers in a suitable solvent system at room temperature. Developed strips were then dried and cut at the solvent front and placed on the layer of surface agar. The dishes were covered and incubated overnight at 37 C.

Plating on MacConkey Agar permitted ready detection of induction. The plaque-forming units contained infective centers of the lysogenic *E. coli* K-12 (λ) consisting of a red colony surrounded by a clear halo of lysis on the colorless colonies of the *E. coli* C600 indicator.

RESULTS AND DISCUSSION

Figure 1 shows a typical bioautograph obtained with the lysogenic test system when doses of

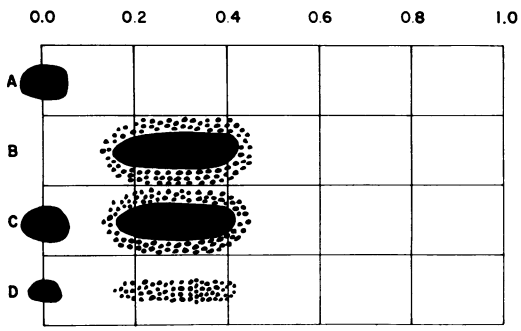

FIG. 1. *Comparative chromatography of paromomycin and mitomycin C. The antibiotics were detected by bioautography on agar seeded for the Escherichia coli K-12 (λ) bacteriophage system. Solvent system: ethyl acetate-methanol (1:1), 6 to 7 hr. The following were used: (A) 40 μg of paromomycin; (B) 25 μg of mitomycin C; (C) an aqueous mixture of 40 μg of paromomycin and 25 μg of mitomycin C; (D) an aqueous mixture of 20 μg of paromomycin and 5 μg of mitomycin C.*

aqueous mitomycin C and paromomycin were applied separately and as a mixture to paper strips. The chromatograms were developed for 6 to 7 hr in a solvent system consisting of ethyl acetate-methanol (11). Mitomycin C migrated on the strip while paromomycin remained at the point of application. Large, well-defined zones of plaque-forming units were obtained with doses of 25 and 5 μg of mitomycin C per strip. Only with the 25-μg dose of mitomycin C per strip was a well-defined zone of antibacterial activity observed. No plaque-forming units were observed around the well-defined antibacterial zones formed at the point of application when 40 and 20 μg of paromomycin were added per strip.

An important feature of the paper chromatographic method is that it permits one to determine whether antibacterial and bacteriophage-induction tests are measuring the same activity in fermentation materials or concentrates. For example, culture NSC-B-158,791, active against Walker intramuscular tumor in rats, also inhibited *Staphylococcus aureus* and was a bacteriophage-inducing agent. To determine whether the antibacterial and bacteriophage-inducing activities were identical, strips were dosed with 400 μg of a crude solid, and duplicate chromatograms were developed for 24 hr in a solvent system of 2-propanol-NH_4OH-$CHCl_3$ (60:40:5) to obtain bacteriophage and *S. aureus* bioautographs. Results are shown in Fig. 2. It was possible to conclude

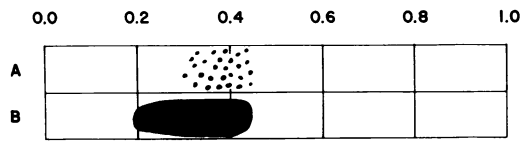

FIG. 2. *Paper chromatography of a crude solid of NSC-B-158,791. Activity was detected by bioautography on agar seeded for the Escherichia coli K-12 (λ) bacteriophage test system (A) and with Staphylococcus aureus (B). Solvent system: 2-propanol-NH_4OH-$CHCl_3$ (60:40:5), developed 24 hr.*

from the paper chromatographic patterns that the *S. aureus* and bacteriophage systems were measuring the same activity and that further work on culture NSC-B-158,791 could be done with either assay system.

Application of the method to systems employing other lysogenic bacteria should be feasible.

ACKNOWLEDGMENTS

We thank P. Watson, Jr., for excellent technical assistance and V. Rossomano and H. Taylor for preparing the paper strips.

This investigation was supported by contract no. PH43-64-1159 from the Cancer Chemotherapy National Service Center, National Cancer Institute.

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