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Bioautography of antibiotic compounds: a simplification and improvement

This note describes a bioautographic procedure for detection of antibiotic activity simplified from that of MEYERS *et al.*[1,2] and improved over that of NICOLAUS *et al.*[3].

The simplifications derive from (1) the use of precoated chromatograms; (2) the elimination of either diffusional transfer of antibiotic onto paper or the overnight diffusional transfer of antibiotic to seeded agar; and (3) the optional omission of the potassium nitrate (0.1 %) added to the medium by MEYERS AND SMITH[1].

The improvements are: (1) the assurance of even thickness of seed agar; (2) the number of separate substances which may be screened simultaneously per chromatographic plate is increased by blocking lateral diffusion; and (3) the method is adaptable to densitometric quantitation.

Briefly, precoated commercial thin-layer chromatographic media (Eastman Chromagram) may be sandwiched between a thin anchoring-layer of gelled nutrient agar (Nutrient Agar, Difco Laboratories, Detroit, Mich.) and an overlying layer of nutrient agar seeded with test organism. The anchoring layer of agar provides a level base for the seeded agar, thus ensuring that the layer of seeded agar will be of uniform thickness.

Experimental

*Material. Escherichia coli** and *Staphylococcus aureus* (ATCC 6538) were used as test organisms. Commercial silica-, alumina-, polyamide- and cellulose-coated chromatographic plates are equally well suited to this technique.

Chromatography. We score the coated surface of the chromatographic plate with a toothpick, graphite pencil or similar tool to produce parallel channels (typically 2 cm wide) and irradiate the plate with UV light for about 1 h to reduce contamination. The samples to be tested are spotted, labeled and developed in the usual manner, taking care to protect the surface of the developed plate from unnecessary contamination. We find it convenient to develop the plate as a whole and, after development, to cut the plate so as to form separate chromatographic strips.

Bioautography. For bioautography with bacteria, 100 ml of base layer of nutrient agar are poured into a sterile Pyrex baking dish nominally 22 × 33 × 4 cm (8¾ × 13 × 1¾ in.) and as the level surface of the agar approaches congelation, the chromatographic strips are gently placed in parallel on the agar surface, coated side up, about 1 cm apart. Care should be taken to avoid trapping air bubbles beneath the strips. The agar is allowed to fully congeal undisturbed and the strips and agar are overlaid with 200 ml of seeded agar. Our seeded agar contains 2 ml of a 24-h culture of the test organism grown in nutrient broth. After the seeded agar has congealed, the chromatographic strips may be isolated from one another by excision of a channel of agar between adjacent strips (Figs. 1 and 2) in order to minimize the lateral diffusion of water-soluble antibiotics. The baking dish is covered with kraft paper and incubated at 37° for 24 h.

* Culture generously supplied by Dr. N. P. WILLETT from the culture collection of his laboratory.

Results

Clear well-defined zones of inhibition of bacterial growth are consistently obtained with active compounds. These are particularly easily visualized by transillumination of the intact bioautogram or of the seeded layer which is easily lifted (Fig. 3) from the chromatogram with the aid of blunt forceps. Such stripping of the seeded layer is facilitated by doubling the volume of seeded agar and the inoculum to provide a thicker and mechanically stronger layer. The stripped agar film may be supported on a glass plate (Fig. 4) and subjected to densitometric analysis. There is very little tendency for particles of the chromatographic substrate to separate from the backing material and adhere to the agar film so as to interfere with a densitometric scan.

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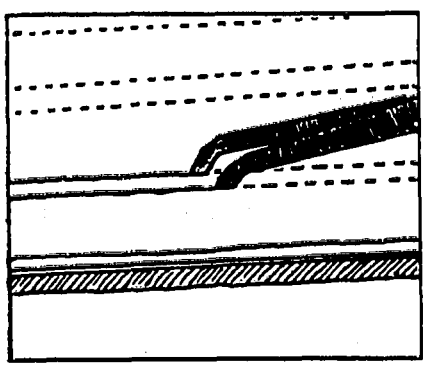


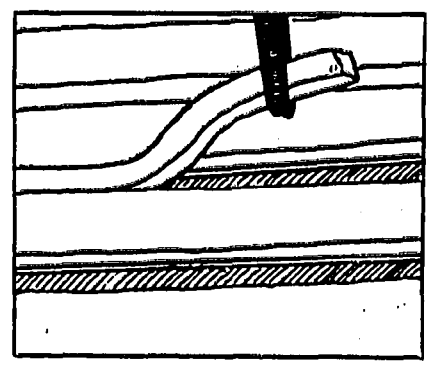
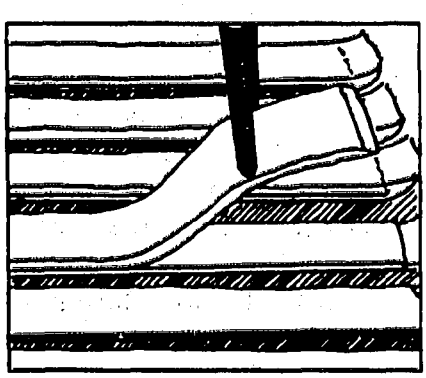


Fig. 1. Cutting of barrier agar channel between chromatographic strips.

Fig. 2. Removal of agar channel.



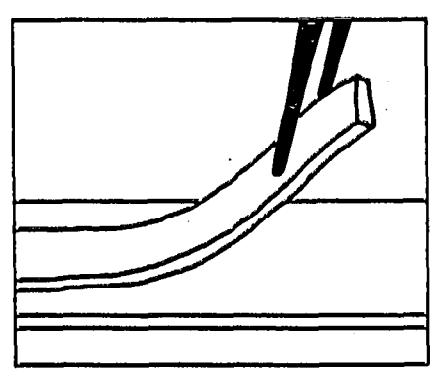


Fig. 3. Lifting of seeded agar from the bioautogram.

Fig. 4. Supporting of seeded agar film on a glass plate for densitometric scan.

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