A simple bioautographic technique for identifying biologically active material on thin-layer chromatograms

During the course of our work on antibiotics (antibacterial and antifungal) we have used thin-layer chromatographic (TLC) techniques for the separation and identification of the active and inactive components and decomposition products. For the location of the biologically active components on the chromatograms, in addition to chemical sprays, we have used a simple bioautographic technique with high sensitivity and reproducibility. Another procedure which has been described earlier is both tedious and involved.[1]

The technique used in this laboratory consists in taking a micro-thin layer of the developed and dried thin-layer chromatograms by pressing a "Scotch Tape" (transparent cellulose adhesive tape) of suitable width on to the thin-layer plate. The Scotch Tape is then carefully removed from the plate and gently tapped on the nonadhesive side to displace loose absorbent material. It is then stretched on a nutrient agar plate seeded freshly with a suitable test organism. Care should be taken to see that the tape is fully stretched without any folds. In order to check whether the tape or the solvent free adsorbent has any effect on the test organism, a similar length of the tape is pressed on to the thin-layer chromatograms at a place where no compound was applied and then is stretched on the seeded plate as a control strip. We found that neither the tape nor any of the adsorbent material had any inhibitory action on the bacterial and fungal test organisms.

The length of time of exposure of the tapes to the seeded plate primarily depends on the nature of the compounds chromatographed. In general polar compounds needed less exposure time than nonpolar ones. Again, those compounds that are highly active need to be exposed for less time than the not so highly active ones. As examples when only 20 units of penicillin had been applied to the chromatogram, and with *Bacillus subtilis* as test organism, the tape was kept in contact with the seeded plate for only 3–5 min; in the case of the antifungal antibiotic hamycin (min. inhibitory concentration to *Candida albicans* is 0.01 μg/ml) only 5 μg were applied to the chromatogram but its time of contact with the Candida seeded plate was 10–15 min, and in some instances the tapes were exposed to the plate for as much as 30 min. When this exposure time is precisely controlled one not only gets a precise and compact spot of inhibition on the plate, but also is able to recognise closely moving compounds that produce a dumb-bell shaped area of inhibition.

The main advantage of this technique is its simplicity as well as using the same thin-layer chromatograms for chemical sprays. Even though we have not tried it in our laboratory, we feel that this method could be used for the identification of biologically stimulatory products using deficiency mutants as test organisms.

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Received October 5th, 1966