without any adverse effect on the separation. The best separation was achieved with a solvent system containing ammonia–methanol–water (5 : 15 : 80). This solvent system also gave good separation of synthetic dyes from a number of samples of fruit jams, fruit jellies, food preserves, canned fruits, beverages and sweet candies.

Discussion

The results indicated that polyamide powder is an excellent material for thin-layer chromatographic separation of synthetic food dyes. Earlier work in our laboratories has also shown the efficiency of polyamide powder for quantitative isolation of synthetic dyes from food materials[1]. Probably the separation is achieved by binding hydrogen bridges between the dyes and the polyamide powder. The natural food dyes usually contain hydroxyl groups (–OH) which form weaker hydrogen bridges, while the synthetic food dyes contain sulfonic acid groups ($-SO_3H$) which form stronger hydrogen bridges, therefore it appears that the elution of natural dyes from the polyamide powder is possible in acidic medium without affecting the binding between the synthetic dyes and the polyamide powder. Since the separation of synthetic dyes depended only on the concentration of the ammonia in the solvent system, the separation of the synthetic dyes may be partly achieved as a result of the difference in the nature and number of functional groups forming hydrogen bridges.

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1 J. DAVÍDEK, *Z. Lebensm. Untersuch. Forsch.*, in press.
2 P. WOHLLENWEBER, *J. Chromatog.*, 7 (1962) 557.
3 C. J. RIEMERSMA AND F. I. M. HESLINGA, *Mitt. Gebiete Lebensm. Hyg.*, 51 (1960) 94.
4 A. MONTAG, *Z. Lebensm. Untersuch. Forsch.*, 116 (1962) 413.
5 J. W. C. PEEREBOOM, *Chem. Weekblad*, 57 (1961) 625.
6 J. DAVÍDEK AND E. DAVÍDKOVA, *Z. Lebensm. Untersuch. Forsch.*, in press.

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Bioautography of antibiotics on thin layer chromatograms

In a previous note[1], we described techniques for the bioautographic detection of antibiotics on thin layer chromatograms. We recommended *Streptococcus lactis* as an assay organism for antibiotics active against Gram positive microorganisms. This bacterium can grow underneath a glass chromatographic plate where oxygen is growth limiting for the more commonly used assay organism, *Staphylococcus aureus* 209P. In subsequent studies however, we found some antibiotics to be non-inhibitory for this bacterium, and hence we were required to use *Staphylococcus aureus* 209P.

As reported previously[1], in order to obtain consistent results with this organism, two overnight incubation periods were required, especially when working with a wide variety of antibiotics with different diffusion characteristics. Essentially, the first overnight incubation period was to allow the antibiotic to diffuse into the agar, and was carried out at 0–4°. The second overnight incubation period, carried out at 37° after removal of the glass chromatographic plate from the agar surface, was to allow growth of the microorganism and thus detection of the bioactive zones. Because of the inconvenience of two overnight incubation periods, we sought modifications of the technique which would allow *Staphylococcus aureus* 209P to grow on an agar surface with reduced levels of oxygen, *i.e.*, under a glass chromatographic plate.

We reasoned that *Staphylococcus aureus* 209P would grow under the conditions required if given a compound capable of replacing oxygen as an oxidant in terminal respiration. Potassium nitrate has been reported to fill this role for various microorganisms[2]. When 0.1 % potassium nitrate was incorporated into both the basal and seed agar layers described in our previous note[1], good growth of the organism occurred under the glass plate. Under these conditions, the organism was as sensitive to a variety of antibiotics as it was when paper chromatograms of the same antibiotics were tested.

Micro Assay Culture Agar (Difco Laboratories, Detroit, Michigan) supplemented with tetrazolium dye (1 ml of an aqueous 2 % (w/v) solution of 2,3,5-triphenyl-2H-tetrazolium chloride per 100 ml of seed layer) can substitute for the basal and seed agar, resulting in better growth of the assay organism. Addition of 0.1 % potassium nitrate to the Micro Assay Culture Agar increases the amount of growth of *Staphylococcus aureus* 209P still further, and gives the most satisfactory results. Excellent contrast between the areas of growth and the zones of inhibition is obtained.

It is apparent that these techniques are easily applicable to adsorbants containing binder or to adsorbants without binder. Most laboratories engaged in antibiotic research are equipped with the materials and cultures required for bioautography. The media are commercially available as dehydrated powders and are easily reconstituted. When working with partially purified antibiotic preparations, U.V. fluorescent and absorbing zones can be marked prior to bioautography and then correlated with bioactive zones. The technique is simple, rapid and well adapted to antibiotic research.

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1 E. MEYERS AND D. A. SMITH, *J. Chromatog.*, 14 (1964) 129.
2 A. NASON, *Bacteriol Rev.*, 26 (1962) 16.

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