CHROM. 3474

Pigment patterns of dermatophytes on thin-layer chromatograms

A number of studies on the pigments isolated from the dermatophytes, as far as their chemical structure and physiological significance are concerned, have been reported[1–23]. Up to date, however, morphological evaluation has incontestably been the dominant criterion for identification in medical mycology; this is the concern of only a small group of specialists.

As a preliminary step in our attempts to obtain a satisfactory identification of pathogenic fungi the criterion of thin-layer chromatography was applied to the pigments isolated from the mycelia of several fungi causing dermatophytosis in human.

Experimental

Pigment extraction. The strains of fungi employed in this work are as follows: *Trichophyton violaceum* 1851 (from Dr. PINETTI of Cagliari University, Italy), *Trichophyton equinum* H-12 (from Dr. SMITH of the Massey University of Manawatu, New Zealand), *Trichophyton megnini* Blanchard IFO-5931 and *Trichophyton tonsurans* Malmsten IFO-5946 (from Institute of Fermentation, Osaka), *Trichophyton rubrum* N-4 and *Trichophyton mentagrophytes* N-3 (from patients), *Microsporum cookei* HUT-2061 (from Dr. OKOSHI of Tokyo University, Tokyo), *Microsporum gypseum* T-1 (from Dr. SUNIT of Chulalonkorn Medical College, Thailand), *Epidermophyton floccosum* IFO-8146 (from the Institute of Fermentation).

The organisms were grown on Sabouraud's dextrose agar, containing 1 % yeast extract, at 28° for 4 weeks. The mycelia were carefully scraped free of culture medium, washed repeatedly with distilled water, freeze-dried and ground in a mortar. After removal of the mycelial lipids with petroleum ether, the total pigments of each of the eight strains were extracted with acetone under a stream of nitrogen. The acetone extracts were evaporated to dryness under reduced pressure and the resulting residues were subjected to thin-layer chromatography.

Thin-layer chromatography. A suspension of 30 g of Kieselgel G (Merck, Germany) in 50 ml of 0.5 *N* oxalic acid solution was homogenized in a mortar, and 10 ml of distilled water were then added. Chromatoplates were prepared by spreading this slurry onto five glass plates (20 × 20 cm) to a thickness of 250 μ using the Yazawa equipment. After drying for 10 min at room temperature, the plates were activated at 110–120° for 45 min and then stored in a desiccator until use. The chromatographic tank was equilibrated for 4–5 h before the plates were developed.

Aliquots of pigment materials were spotted in chloroform solution at the origin, 2.5 cm from the bottom of the plates. Ascending chromatography was carried out at room temperature with a solvent system consisting of benzene–acetone (3:1, v/v) for 25 min at which time the solvent front had advanced about 15 cm. The plates were removed and dried in air and the procedure was repeated using the same solvent. The chromatographed plate was then sprayed with 2 *N* sodium hydroxide solution for visualization.

Results and discussion

Although various spot patterns were found on the TLC plate before spraying

with alkali, among the eight strains tested the major components were those such as xanthomegnin[12,14,19] (yellow, R_F 0.44), aurosporin (yellow, R_F 0.54), citrosporin (orange, R_F 0.32), violosporin (violet, R_F 0.39) and rubrosporin (red, R_F 0.10), which were most distinct in *Microsporum cookei* HUT-2061 and *Trichophyton violaceum* 1851. Xanthomegnin has already been identified as (—)3,3′-bis[2-methoxy-5-hydroxy-7-(2-hydroxypropyl)-8-carboxy-1,4-naphthoquinone lactone] by JUST *et al.*[12], and work on the chemical structures of the latter four pigments is now in progress.

In Fig. 1 a photograph of the chromatogram after alkali treatment is shown; almost all of the yellow spots turned to purple except one pale yellow spot from *Epidermophyton floccosum* IFO-8146 which changed to bright yellow instead, while the yellow spots with R_F values above 0.7 remained unchanged.

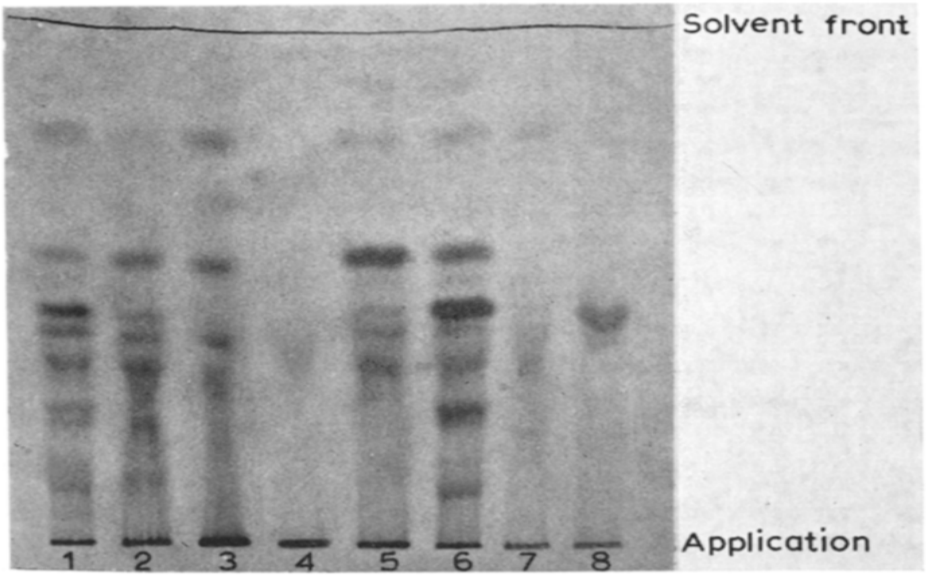

Fig. 1. Photograph of thin-layer chromatogram sprayed with 2 *N* sodium hydroxide to reveal the pigments extracted from dried mycelia of several strains of dermatophytes. Adsorbent: Silica Gel G (Merck); developing solvent system: benzene–acetone (3:1). 1 = *Trichophyton violaceum* 1851; 2 = *Trichophyton rubrum* N-4; 3 = *Trichophyton mentagrophytes* N-3; 4 = *Trichophyton tonsurans* IFO-5946; 5 = *Trichophyton equinum* H-12; 6 = *Microsporum cookei* HUT-2061; 7 = *Microsporum gypseum* T-1; 8 = *Epidermophyton floccosum* IFO-8146.

It is clear that with TLC *Epidermophyton floccosum* IFO-2061, *Microsporum gypseum* T-1 and *Trichophyton tonsurans* IFO-5946 can be distinguished from other strains showing similar, but not identical pigment patterns. It was, however, shown on the basis of some results such as color reactions with alkali, magnesium acetate in methanol, pH indicator property, reversible oxidation–reduction phenomena, and U.V. and I.R. spectra that almost all of these pigments are quinoid, possibly of the naphthoquinone type.

Since preliminary experiments have shown that the age of culture and type of medium has no particular effect upon the distribution pattern of the pigments, it is suggested that the variability in the spot pattern on TLC is due to the differences in metabolites from the mycelia strains themselves. Therefore, it is felt that thin-

layer chromatography could be a useful criterion in the classification of dermatophytes in place of morphological identification.

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Received February 20th, 1968

CHROM. 3536

Artefacts arising from the use of sucrose solutions in electrofocusing techniques

Electrofocusing, pioneered by VESTERBERG AND SVENSSON[1–4], is a relatively new technique for the separation of protein mixtures. It involves the concentration of the proteins at their isoelectric points along a pH gradient by means of high voltage. A feature of the method is the use of a density gradient along the buffer tube to avoid diffusion of the bands by thermal agitation. The most commonly used commercial instrument is similar to that of the original workers and is manufactured by L.K.B. Ltd., the density gradient being formed usually from sucrose solutions.

Using this instrument, it has been found that many samples of "Analar" sucrose contain impurities which focus along the pH gradient in an identical manner to