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Simple Medium for Pigment Production by the Erythrasma Diphtheroid

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A simple method for the isolation and identification of the causative diphtheroid of erythrasma, *Corynebacterium minutissimum*, utilizes Muiller-Hinton Agar.

The demonstration by Sarkany et al. (2, 3) that erythrasma is a bacterial infection of the stratum corneum caused by a cultivable diphtheroid has made a simple method for isolating and identifying the causative organism desirable. These workers found that the organism grew on a variety of media, but pigment production was irregular on chocolate-agar, sheep blood-agar, or yeast extract-casein-agar and was only reliable on a special preparation of tissue culture medium no. 199 with 20% fetal bovine serum. Further, the phenol red indicator of medium no. 199 could interfere with detection of the fluorescence of the erythrasma diphtheroid, since acid production by a variety of organisms could result in strong yellow fluorescence of the indicator. In addition, some batches of fetal bovine serum were inhibitory to growth of the diphtheroid.

Because of the very prominent pigment production of fluorescing *Pseudomonas aeruginosa* plated on Mueller-Hinton Agar, presumably as a result of the iron content of the medium (1), we attempted to detect fluorescence of the erythrasma diphtheroid on Mueller-Hinton Agar plates, which are readily available in many diagnostic laboratories not otherwise specially equipped for dermatological bacteriology (in our laboratory the medium is used routinely for disc sensitivity testing), instead of on the complex medium originally found optimal by Sarkany et al. (3).

A patient with the clinical diagnosis of erythrasma, based upon the gross appearance of the lesions and their pink fluorescence under Wood's light illumination, was studied. Cotton swabs from lesions between the patient's toes were plated directly upon Trypticase Soy Agar (BBL) with 10% defibrinated sheep blood, fresh beef heart infusion agar with 10% defibrinated sheep blood, chocolate-agar (Proteose Peptone Agar Base with Supplement B, Difco), Mueller-Hinton Agar (Difco), and King's Media A and B (Difco), and the swabs were placed in Robertson's cooked meat medium and in N I H Fluid Thioglycollate Medium (Difco). Replicate cultures were incubated at 30 and at 37 C aerobically, in and out of a candle jar, and anaerobically (Gaspak, BBL) and were held for 5 days. Isolates were examined for ability to ferment dextrose, lactose, sucrose, and maltose in 1% concentration in peptone water with Vaspar seals, and were held at 30 and at 37 C for 21 days.

For comparison, a stock strain of *Corynebacterium minutissimum* was examined. Fluorescence was induced with an ultraviolet light (Blak-Ray, UVL-22, 320 to 400 nm; Ultra-violet Products, San Gabriel, Calif.).

Organisms producing bright pink fluorescence under ultraviolet illumination were detected upon all solid media except for King's A and B, but growth was better at 30 C than at 37 C and was less marked under anaerobic conditions than in an air or candle jar, in the latter two of which growth appeared about equal. In all cases, the fluorescence was difficult to see and was limited to the colony, except on the Mueller-Hinton agar on which extensive pigment production occurred by the 2nd day of incubation. This pigment, in both the new isolate and the stock strain, was extracted with water; on spectrofluorometric examination, it showed a peak at 700 nm when activated with light at 365 nm, differing slightly from the maximum at 613 nm found by others (3). Although pigmentation was noted to fade somewhat by the 6th day of culture, readily detectable pigment continued to be present for a period as long as 15 days. This is in contrast to the reported experience of Sarkany et al. (3) with medium no. 199, in which the pigment faded after about 48 hr.

Fermentation tests of two isolates from the patient showed acid production in glucose and maltose (4 days) and sucrose (12 days) for a large-colony form and no sucrose fermentation in what appeared to be a small-colony variant. Ability to

ferment sucrose was lost on subculture. Four other isolates, all producing similar pigment, did not ferment sugars, nor did the reference organism.

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