Rapid Identification of the Taxon *Rhodochrous* in the Clinical Laboratory

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Degradation of ethylene glycol in Middlebrook 7H-10 agar medium by 26 out of 29 strains of the taxon *rhodochrous* seems to permit its separation from rapidly growing mycobacteria and certain actinomycetes which only exceptionally showed this property.

Gordon (4) considered the taxon *rhodochrous* to be an intermediate between nocardiae and mycobacteria. Only recently Goodfellow and co-workers (1, 2) were able to differentiate between this taxon and the genus *Mycobacterium* as well as the genus *Nocardi*a by employing numerical taxonomy. Without the facility to perform a wide variety of tests within the framework of numerical taxonomy on an occasional acid-fast or semi-acid-fast organism, the clinical laboratory finds itself in a dilemma if the question of clinical significance of such isolates arises.

Some nocardiae and rapidly growing mycobacteria are considered potential human pathogens, whereas no pathogenicity has yet been reported, to our knowledge, about the taxon *rhodochrous*. Routine drug susceptibility tests in Middlebrook 7H-10 agar showed earlier that some of the pigmented, semi-acid-fast isolates degrade ethylene glycol (mol wt 62.07), which is used at a concentration of not more than 1% as an organic solvent for the incorporation of water-insoluble antituberculous drugs into culture media (6). The degradation of ethylene glycol by microorganisms appears as an extensive milky turbidity in the almost clear 7H-10 medium, provided ethylene glycol was added to the agar after autoclaving.

To utilize this phenomenon possibly for taxonomic purposes, we investigated in a double blind study 75 strains from the mycobacteriology section and the mycology branch of the Center for Disease Control (CDC), 4 strains from the Massachusetts State Laboratory, and 10 strains of our own isolates. The strains from CDC consisted of five strains each of *M. smegmatis*, *M. phlei*, and the taxon *rhodochrous*, as well as 13 strains of unidentified mycobacterial rapid growers, three strains of *M. flavescens*, and six strains of *M. fortuitum*. All of which were obtained from the stock culture collection of the mycobacteriology section through the courtesy of R. E. Beam. Another 22 strains of the taxon *rhodochrous* were obtained from the same source. Four strains each of *Nocardia asteroides*, *Nocardia brasiliensis*, *Nocardia caviae*, and two strains each of *Actinomadura dassonvillei* and *Actinomadura madurae* were obtained through the courtesy of William A. Causey of the mycology branch at CDC. Another four strains of *N. asteroides* were obtained through the courtesy of Marion Holmes of the Massachusetts State Laboratory Institute. No attempt was made to confirm the identification of strains received from either Federal or State Reference Laboratories. The 10 strains isolated by this laboratory were identified by conventional methods (3, 5). Five were identified as rapidly growing mycobacteria, three as *N. asteroides*, and two as *rhodochrous*. The identification of the latter was made chiefly on the grounds of morphology and pigmentation. All 89 strains were subcultivated in Middlebrook 7H-9 broth and incubated for 5 to 10 days at 37°C. A dilution of 1:10 in sterile distilled water containing 0.01% Tween 80 (polyethylene sorbitan monooleate) was made after growth had occurred, and 0.25 ml of the dilution was inoculated onto 7H-10 medium with and without 1% of ethylene glycol in a two-section 100-mm plastic petri dish. Direct transfer of inoculum from solid medium onto 7H-10 medium with and without ethylene glycol by loop was performed also; however, the results were more consistent if dilutions of 7H-9 broth were used as inoculum. After 5 to 10 days of incubation at 37°C without CO₂ both sections of 7H-10 medium showed confluent growth, but the section containing ethylene glycol showed a considerable milky turbidity which was caused by 26 out of 29 *rhodochrous* strains tested (90%)
and co-workers (2) permits the separation of rhodochrous from rapidly growing mycobacteria and nocardiae with the same degree of reliability as does the ethylene glycol test.

In conclusion, the incorporation of 1% ethylene glycol into the 7H-10 medium seems to permit the separation of the taxon rhodochrous from nocardiae and rapidly growing mycobacteria with an accuracy of 90%. This test puts a clinical laboratory in a better position to decide whether an acid-fast or semi-acid-fast, mostly pigmented, and rapidly growing organism belongs to either potentially pathogenic mycobacterial rapid growers and nocardiae or the taxon rhodochrous, on which no pathogenicity has yet been reported.

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(FIG. 1. Differentiation of cultures of nocardiae and rhodochrous by ethylene glycol in 7H-10 medium. Note turbidity in section of rhodochrous plate containing ethylene glycol.)

TABLE 1. Ethylene glycol degradation reactions by the taxon rhodochrous, rapidly growing mycobacteria, and aerobic actinomycetes

| Species                  | Total no. of strains | No. of strains giving: | Positive reaction | Negative reaction |
|--------------------------|----------------------|------------------------|-------------------|-------------------|
| Taxon rhodochrous        | 29                   | 26                     | 3                 |
| Mycobacterium smegmatis  | 5                    | 1                      | 4                 |
| M. phlei                 | 5                    | 0                      | 5                 |
| M. fortuitum             | 6                    | 0                      | 6                 |
| M. sp. rapid             | 18                   | 0                      | 18                |
| M. flavescens            | 3                    | 0                      | 3                 |
| Nocardia asteroides      | 11                   | 2                      | 9                 |
| N. brasiliensis           | 4                    | 0                      | 4                 |
| N. cavia                 | 4                    | 0                      | 4                 |
| Actinomadura dassonville | 2                    | 0                      | 2                 |
| A. madurae               | 2                    | 0                      | 2                 |

(Fig. 1 and Table 1). Nocardiae and rapidly growing mycobacteria grew equally well on 7H-10 with and without ethylene glycol but showed this phenomenon only exceptionally under the same experimental conditions. Only two strains of N. asteroides and one strain of M. smegmatis behaved like cultures of rhodochrous in the ethylene glycol test (Table 1).

Although numerical taxonomy appears to be the answer for the classification of microbial organisms, clinical laboratory tests must be designed to obtain pertinent data for a clinical diagnosis in a relatively short period of time. None of the 170 characters listed by Goodfellow and co-workers (2) permits the separation of rhodochrous from rapidly growing mycobacteria and nocardiae with the same degree of reliability as does the ethylene glycol test.

In conclusion, the incorporation of 1% ethylene glycol into the 7H-10 medium seems to permit the separation of the taxon rhodochrous from nocardiae and rapidly growing mycobacteria with an accuracy of 90%. This test puts a clinical laboratory in a better position to decide whether an acid-fast or semi-acid-fast, mostly pigmented, and rapidly growing organism belongs to either potentially pathogenic mycobacterial rapid growers and nocardiae or the taxon rhodochrous, on which no pathogenicity has yet been reported.

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