Identification of *Mycobacterium kansasii* by Susceptibility to Hydroxylamine

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Among biochemical tests useful in the study of mycobacteria, the hydroxylamine test is shown to be an effective tool in the differentiation of *Mycobacterium kansasii* from saprophytic strains of group III acid-fast bacilli when combined with the photochromogen test.

Members of Runyon's four groups of atypical acid-fast bacilli (4) differ greatly in their virulence for man. Since *Mycobacterium kansasii* is markedly pathogenic for man and the Tween 80-positive members of Runyon's group III, which may resemble *M. kansasii* are not, a definitive differential test is essential. The photochromogen test is, of course, the major tool but may be falsely negative on initial performance unless careful attention is given to detail. Several biochemical tests which might serve as an adjunct to the photochromogen test were investigated. Of these, the hydroxylamine test proved to be an excellent auxiliary test.

The strains tested were either isolated from specimens sent to the Division or were submitted for identification by local laboratories. One, 2409-67, was received from E. Runyon (Veterans Administration Hospital, Salt Lake City, Utah).

The following biochemical tests were used: the Tween 80 utilization test described by L. G. Wayne (6), the catalase test at 68 C described by A. Andrejew et al. (1) and G. P. Kubica et al. (3), the nitrate reduction as described by G. P. Kubica (2), photochromogenesis, and the hydroxylamine test.

For photochromogenesis, Löwenstein-Jensen medium inoculated with the unknown strain is incubated in the dark at 37 C for 7 to 10 days. When good growth develops, a portion of the slant is covered with black paper and the tube is exposed for 2 hr to light from a 60-w bulb at a distance of approximately 18 inches (45.72 cm). The cultures are reincubated for an additional 48 hr and examined for change in color from buff to yellow-orange in the portion of the slant that has been exposed to the light.

For the hydroxylamine test (5), one drop of a suspension of acid-fast bacilli equal in density to a no. 1 MacFarland turbidity standard is placed with a Pasteur pipette in duplicate tubes of Löwenstein-Jensen medium with and without 500 µg of hydroxylamine (J. T. Baker Chemical Co., Phillipsburg, N.J.) per ml. The hydroxylamine is added prior to sterilization of the medium. The tubes are incubated at 37 C and examined weekly for up to 4 weeks for the presence of growth.

Seventeen strains of *M. kansasii* and 31 strains of the saprophytic subgroups within Runyon's Group III received during this study were examined. The results with representative strains are given in Table 1; the other strains resembled 3364-66 or 2969-66.

All of the cultures that yielded positive results in the photochromogen test did not grow on hydroxylamine medium within a 2-week period after growth appeared on the controls, whereas all strains that grew on hydroxylamine (all of the *M. terrae* strains and "V" strains tested) were negative in the photochromogen test. Of the 52 cultures tested, only 2 were negative in both tests; 3691-61 proved to be a strain of *M. gastri*, differing from *M. kansasii* in other reactions, whereas 2409-67, received from Runyon as a nonphotochromogenic *M. kansasii*, was nitrate-positive and had the typical colony morphology of the photochromogenic strains. Although the number reported is small, additional studies have confirmed these findings.

When the acid-fast microorganisms isolated grow well at room temperature, are catalase-positive at 68 C, hydrolyze Tween 80, do not grow on hydroxylamine medium, and are not photochromogenic, the results are considered incompatible for identification and the photochromogen test is repeated. Because the photochromogen test was initially negative, strain 1007-66 included with 3364-66 in Table 1 was first classified as a group III acid-fast bacillus, positive for Tween 80 utilization. However, after a negative response on hydroxylamine medium and repeated photo-
Table 1. Results of biochemical tests

| Representative strain number | Number of strains resembling this strain | Hydroxylamine | Nitrate reduction | Catalase at 68°C | Photochromogen | Final identification |
|-------------------------------|----------------------------------------|---------------|-------------------|------------------|----------------|----------------------|
| 3364-66                       | 16                                     | −             | 3−5−              | +                | +              | Mycobacterium kansasii |
| 2409-67                       | 1                                      | −             | 4−                | −                | −              | M. kansasii          |
| 2969-66                       | 27                                     | +             | 2−3−              | −                | −              | M. terrae           |
| 723-66                        | 4                                      | +             | 1−                | −                | −              | "V" bacillus         |
| 6874-60                       | 2                                      | +             | 5−                | −                | −              | "V" bacillus         |
| 3691-61                       | 2                                      | −             | 1−                | −                | −              | M. gastri           |

chromogenic studies, which were then found to be positive, it was reclassified as *M. kansasii*. Only in these rare situations, when the hydroxylamine test is negative and photochromogenicity is repeatedly lacking, are other studies required. These include colony morphology, nitrate reduction, and arylsulfatase activity.

**LITERATURE CITED**

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