Differential Identification of _Mycobacterium fortuitum_ and _Mycobacterium chelonei_

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_Mycobacterium fortuitum_ and _Mycobacterium chelonei_ are distinguished unambiguously by the combined use of five test characters: nitrate reductase, β-glucosidase, acid production from fructose, penicillinase, and trehalase. Typically, _M. fortuitum_ was nitrate reductase positive, β-glucosidase positive, penicillinase negative, and trehalase negative and produced acid from fructose; _M. chelonei_ was nitrate reductase negative, β-glucosidase negative, penicillinase positive, and trehalase positive and did not produce acid from fructose.

There is increasing awareness of human infections caused by organisms of the _Mycobacterium fortuitum_ complex, particularly as agents of infections complicating surgery (for a comprehensive and recent review, see reference 12). Detailed epidemiological or ecological investigations pertinent to the understanding of these infections and to their prevention require that the laboratory be capable to distinguish unambiguously the species that are recognized to be part of the _M. fortuitum_ complex. However, numerical taxonomy studies (4) have shown that the test characters that discriminate two major taxa (_M. fortuitum_ and _Mycobacterium chelonei_) do not always appear satisfactory to place individual clinical isolates into one taxon or the other. It is the purpose of this report to show that the combined use of five tests allows the clear separation of these bacteria into two major groups, one corresponding to _M. fortuitum_ and the other to _M. chelonei_.

**MATERIALS AND METHODS**

_Mycobacterial strains_. A total of 355 strains of nonpigmented, rapidly growing mycobacteria that formed an arylsulfatase (3-day arylsulfatase test; 11) have been received in this laboratory for identification since 1978, and these strains were used in this study. In addition the following stock strains of rapidly growing mycobacteria were examined: _M. fortuitum_ (ATCC 6841) and _Mycobacterium peregrinum_ (sic) (ATCC 14467). Among the representatives identified as _M. chelonei_ subsp. _chelonei_ were _Mycobacterium friedmannii_ (sic) (NCTC 946 = ATCC A9235) and _Mycobacterium borstelense_ (sic) (SN 165 = ATCC 19237), and strains known as _M. chelonei_ subsp. _abscessus_ included _Mycobacterium abscessus_ (sic) (ATCC 19977), _M. chelonei_ subsp. _abscessus_ (Stanford no. 133), and _Mycobacterium runyonii_ (sic) (ATCC 14472). Other stock strains included _Mycobacterium smegmatis_ (ATCC 14468), _Mycobacterium phlei_ (ATCC 11758), _Mycobacterium vaccae_ (SN 901), _Mycobacterium parafortuitum_ (ATCC 19686), and _Mycobacterium dierhoferi_ (ATCC 19340).

**Test procedures.** All strains, including wild-type strains and the indicated stock strains, were studied using the 3-day arylsulfatase and nitrate reductase tests as described by Vestal (11), acid production from fructose as described by Gordon and Smith (3), and the β-glucosidase test as described by David and Jahan (2).

The penicillinase and trehalase tests, developed only recently, were used to test 74 recent isolates and the indicated representative strains as well. The tests were performed as follows. Actively growing cultures on Lowenstein-Jensen slants were used for testing. Samples of from 5 to 10 mg of bacterial mass, scraped from the surface of actively growing cultures on Lowenstein-Jensen medium slants, were transferred to screw-cap tubes (16 by 125 mm) containing 0.5 ml of the appropriate substrate. For the penicillinase activity determination, the substrate was a 10⁻¹ M solution (35.65 mg/ml) of penicillin G (Sigma Chemical Co., St. Louis, Mo.) in 0.05 tris(hydroxymethyl)aminomethane buffer (pH 8.0) containing 0.01% phenol red indicator (10). The reaction mixtures were incubated at 37°C for 5 h, and the results were scored. The change of the indicator to distinct bright yellow indicated a positive reaction. For trehalase (6) determination, the substrate was a 10⁻¹ M solution of trehalose (O-1-α-glucopyranosyl-α-D-glucopyranoside) (Sigma) in 0.05 M tris(hydroxymethyl)aminomethane buffer (pH 7.0). The reaction mixtures were incubated at 37°C for 5 h, at which time the presence of glucose was revealed by adding 0.5 ml of phosphoglucooxidase enzyme color reagent prepared as recommended by the manufacturer (Sigma). After addition of the reagent, the mixtures were replaced at 37°C for 30 min. The development of a brown color indicated the presence of glucose and, therefore, a positive reaction.

**RESULTS**

The phenotypes of the representative strains of various rapidly growing mycobacteria are in-
dicated in Table 1. With reference to the test results obtained using the nitrate reductase test, the $\beta$-glucosidase test, and the test for production of acid from fructose, the majority (318, or 90%) of the strains isolated from clinical specimens were divided into two groups: one, corresponding to \textit{M. fortuitum}, comprised 202 strains (57%) that were positive in all three tests; the other, corresponding to \textit{M. chelonei} subsp. \textit{chelonei} and \textit{M. chelonei} subsp. \textit{abscessus}, included 116 strains (33%) that were negative in all three tests (Table 2, first evaluation). Because the \textit{M. chelonei} strains were characterized by negative reactions, we searched for other properties of these organisms that were mutually exclusive for \textit{M. fortuitum}. As shown in Table 1, \textit{M. chelonei} subsp. \textit{chelonei} and \textit{M. chelonei} subsp. \textit{abscessus} formed penicillinase and trehalase, whereas \textit{M. fortuitum} did not. According to the results shown in Table 2 (second evaluation), 66 (89%) of the strains had one of two sets of characteristics: \textit{M. fortuitum} was nitrate reductase and $\beta$-glucosidase positive and penicillinase and trehalase negative and produced acid from fructose; \textit{M. chelonei} was nitrate reductase and $\beta$-glucosidase negative and penicillinase and trehalase positive and did not produce acid from fructose. The remaining strains (8 strains, or 11%) differed from \textit{M. fortuitum} by only one test character ($\beta$-glucosidase), and their inclusion in this taxon was possibly justifiable. On the other hand the stock strain of \textit{M. chelonei} subsp. \textit{abscessus}, designated as \textit{M. runyoni} ATCC 14472, differed from \textit{M. chelonei} also by one test character (5- and 18-h trehalase). Considering that \textit{M. runyoni} clustered with \textit{M. abscessus} in numerical taxonomy investigations (4), the negative result in the trehalase test may indicate that this strain is a variant of \textit{M. chelonei} subsp. \textit{abscessus}. Also, the strain designated \textit{M. borstelense} ATCC 19237 did not give a positive 5-h trehalase test. Whether the deviations from the main phenotypes that were found in occasional strains were of taxonomic significance, or might be useful as epidemiological markers, could not be assessed because the number of strains studied is still limited.

Of the strains indicated in Table 2, 32 were isolated from clinical specimens, and 42 were isolated from water samples. Organisms belonging to both taxa were isolated from either source, and it is interesting that \textit{M. chelonei} does not seem to have been isolated from water before (12).

The test procedures that were evaluated also appeared to have diagnostic value when applied to other rapidly growing mycobacteria; however, the number of strains assembled for this study

| Table 1. Expected results of \textit{M. fortuitum}, \textit{M. chelonei}, and other rapid growers with respect to six test characters |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Group*            | Representative strains: designation (no.)* | 3-Day arylsulfatase | Nitrate reductase | $\beta$-Glucosidase | Acid from fructose | Trehalase         |
| \textit{M. fortuitum} | \textit{M. fortuitum} (ATCC 6841) | + | + | + | - | - | 0 |
|                  | \textit{M. peregrinum} (ATCC 14467) | + | + | + | - | - | 0 |
| \textit{M. chelonei} subsp. \textit{chelonei} | \textit{M. friedmanii} (NCTC 946) | + | - | - | + | + | + |
|                  | \textit{M. borstelense} (ATCC 19237) | + | - | - | + | + | + |
| \textit{M. chelonei} subsp. \textit{abscessus} | \textit{M. abscessus} (ATCC 19977) | + | - | - | + | + | + |
|                  | \textit{M. runyoni} (ATCC 14472) | + | - | - | + | + | + |
|                  | \textit{M. chelonei} (JS 133) | + | - | - | + | + | + |
| Other rapidly growing mycobacteria | \textit{M. smegmatis} (ATCC 14468) | - | + | - | + | + | - |
|                  | \textit{M. phlei} (ATCC 11758) | - | + | - | + | + | - |
|                  | \textit{M. vaccae} (SN 901) | - | + | + | - | - | - |
|                  | \textit{M. parafortuitum} (ATCC 19686) | - | + | + | - | - | - |
|                  | \textit{M. diernhoferi} (ATCC 19340) | - | + | + | + | - | - |

* Nomenclature and synonym as described in \textit{Berger's Manual of Determinative Bacteriology} (7).

* ATCC, American Type Culture Collection; NCTC, National Centre of Type Culture (U.K.); JS, John Stanford; SN, from R. Bönich.}

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was too small for conclusions concerning their discriminative power in the identification of the nonpathogenic rapid growers.

**DISCUSSION**

The nonpigmented rapidly growing mycobacteria that form an arylsulfatase (3-day test) and can grow on MacConkey agar without crystal violet (M. fortuitum complex), when analyzed by means of numerical taxonomy, formed two major clusters (4). One of the clusters contained the taxa *M. fortuitum* and *M. peregrinum*, and the other contained the taxa *M. chelonei* and *M. abscessus*. Because *M. fortuitum-M. peregrinum*, as well as *M. chelonei-M. abscessus*, were so closely related, Kubica et al. (4) suggested the use of the specific names *M. fortuitum* and *M. chelonei* to designate the main clusters and the use of subspecific designations to refer to the subspecies of these taxa. *M. peregrinum* may be a synonym of *M. fortuitum* (1, 4, 7, 8), whereas in *M. chelonei* one can recognize the subspecies *M. chelonei* subsp. *abscessus* and *M. chelonei* subsp. *chelonei* (1, 4, 7, 9). According to the results of the present study, the phenotypes of *M. fortuitum* and *M. chelonei* are mutually exclusive with respect to five test characters; however, the respective subspecies could not be differentiated.

Four of the tests proposed in this report were rapid tests that can be performed and read the same day, and therefore they are useful in the clinical laboratory. The deletion of the test for acid production from fructose from the set did not decrease the diagnostic ability of the set.

Because these tests are known to have diagnostic value in other areas of mycobacteriology (2, 5, 10) they were included in our regular reference activities with the purpose of assessing their relative importance within a general scheme for the identification of mycobacteria.

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**LITERATURE CITED**


