

Differential Identification of *Mycobacterium fortuitum* and *Mycobacterium chelonae*

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Mycobacterium fortuitum and *Mycobacterium chelonae* 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. chelonae* 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 chelonae*) 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. chelonae*.

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. chelonae* subsp. *chelonae* were *Mycobacterium friedmanii* (sic) (NCTC 946 = ATCC A9235) and *Mycobacterium borstelense* (sic) (SN 165 = ATCC 19237), and strains known as *M. chelonae* subsp. *abscessus* included *Mycobacterium abscessus* (sic) (ATCC 19977), *M. chelonae* subsp. *abscessus* (Stanford no. 133), and *Mycobacterium runyoni* (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 diernhoferi* (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 phosphoglucosidase 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-

indicated in Table 1. With reference to the test results obtained using the nitrate reductase test, the β -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 *M. fortuitum*, comprised 202 strains (57%) that were positive in all three tests; the other, corresponding to *M. chelonae* subsp. *chelonae* and *M. chelonae* subsp. *abscessus*, included 116 strains (33%) that were negative in all three tests (Table 2, first evaluation). Because the *M. chelonae* strains were characterized by negative reactions, we searched for other properties of these organisms that were mutually exclusive for *M. fortuitum*. As shown in Table 1, *M. chelonae* subsp. *chelonae* and *M. chelonae* subsp. *abscessus* formed penicillinase and trehalase, whereas *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: *M. fortuitum* was nitrate reductase and β -glucosidase positive and penicillinase and trehalase negative and produced acid from fructose; *M. chelonae* was nitrate reductase and β -glucosidase negative and penicillinase and trehalase positive and did not produce acid from fructose. The remaining strains (8 strains, or 11%) differed from *M. fortuitum* by only one test

character (β -glucosidase), and their inclusion in this taxon was possibly justifiable. On the other hand the stock strain of *M. chelonae* subsp. *abscessus*, designated as *M. runyoni* ATCC 14472, differed from *M. chelonae* also by one test character (5- and 18-h trehalase). Considering that *M. runyoni* clustered with *M. abscessus* in numerical taxonomy investigations (4), the negative result in the trehalase test may indicate that this strain is a variant of *M. chelonae* subsp. *abscessus*. Also, the strain designated *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 *M. chelonae* 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 *M. fortuitum*, *M. chelonae*, and other rapid growers with respect to six test characters

Group ^a	Representative strains: designation (no.) ^b	Test results						
		3-Day arylsul-fatase	Nitrate reduc-tase	β -Glu-cosidase	Acid from fruc-tose	Penicil-linase	Trehalase	
							5 h	18 h
<i>M. fortuitum</i>	<i>M. fortuitum</i> (ATCC 6841)	+	+	+	+	-	-	0
	<i>M. peregrinum</i> (ATCC 14467)	+	+	+	+	-	-	0
<i>M. chelonae</i> subsp. <i>chelonae</i>	<i>M. friedmanii</i> (NCTC 946)	+	-	-	-	+	+	+
	<i>M. borstelense</i> (ATCC 19237)	+	-	-	-	+	-	+
<i>M. chelonae</i> subsp. <i>absces-sus</i>	<i>M. abscessus</i> (ATCC 19977)	+	-	-	-	+	+	+
	<i>M. runyoni</i> (ATCC 14472)	+	-	-	-	+	-	-
	<i>M. chelonae</i> (JS 133)	+	-	-	-	+	+	+
Other rapidly growing myco-bacteria	<i>M. smegmatis</i> (ATCC 14468)	-	+	-	+	+	-	-
	<i>M. phlei</i> (ATCC 11758)	-	+	-	+	-	-	-
	<i>M. vaccae</i> (SN 901)	-	+	+	+	-	-	-
	<i>M. parafortuitum</i> (ATCC 19686)	-	+	+	+	-	-	-
	<i>M. diernhoferi</i> (ATCC 19340)	-	+	+	+	+	-	-

^a Nomenclature and synonym as described in *Bergey's Manual of Determinative Bacteriology* (7).

^b ATCC, American Type Culture Collection; NCTC, National Centre of Type Culture (U.K.); JS, John Stanford; SN, from R. Bönicke.

TABLE 2. Distribution of strains of the *M. fortuitum* complex isolated from clinical specimens and water samples

Species identification ^a	First evaluation ^b					Second evaluation ^b					
	No. of strains in year:			Total no. of strains	Frequency (%)	Clinical specimens		Water samples		Total	Frequency (%)
	1978	1979	1980			No.	Frequency (%)	No.	Frequency (%)		
<i>M. fortuitum</i>	92	77	33	202	57	15	47	30	84	45	61
<i>M. chelonae</i>	38	29	49	116	33	15	47	6	8	21	28
Unidentified	22	10	5	37	10	2	6	6	8	8	11

^a *M. fortuitum* = Strains exactly nitrate reductase, β -glucosidase, and acid production (from fructose) positive in the first evaluation, and exactly the same plus penicillinase and trehalase negative in the second evaluation. *M. chelonae* = Strains exactly nitrate reductase, β -glucosidase, and acid production negative in the first evaluation, and exactly the same plus penicillinase and trehalase positive in the second evaluation. Unidentified = Strains that differed by a single character from the first two groups.

^b In the first evaluation, the determination was based on the results of tests for nitrate reductase, β -glucosidase, and production of acid from fructose. As indicated in the text, this evaluation was done before the additional penicillinase and trehalase tests were developed. The second evaluation included all five test procedures.

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. chelonae* and *M. abscessus*. Because *M. fortuitum*-*M. peregrinum*, as well as *M. chelonae*-*M. abscessus*, were so closely related, Kubica et al. (4) suggested the use of the specific names *M. fortuitum* and *M. chelonae* 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. chelonae* one can recognize the subspecies *M. chelonae* subsp. *abscessus* and *M. chelonae* subsp. *chelonae* (1, 4, 7, 9). According to the results of the present study, the phenotypes of *M. fortuitum* and *M. chelonae* 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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