Mycobacterium Resembling *Mycobacterium fortuitum* That Produces Brown Pigment

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Two cultures of acid-fast bacilli with characteristics most closely resembling those of *Mycobacterium fortuitum* were recovered as casual isolates from sputa of a patient with an apparent bronchogenic tumor. One of the cultures was consistently cream colored to rosy buff. The other, however, changed from buff to rust to dark brown and had the gross appearance of a fungus culture.

Two cultures of acid-fast bacilli from sputa of a patient with an apparent bronchogenic tumor were submitted to this laboratory for identification by a Veterans Administration (VA) Hospital in the Northeastern United States. The organisms appeared to be casual isolates, since the referring laboratory reported that only a few colonies of growth appeared initially and subsequent specimens were negative.

When received on Lowenstein-Jensen (L-J) medium, subculture 1181 had rough, very dark brown growth that on gross examination appeared more typical of a fungus than a *Mycobacterium*. On the other hand, subculture 1182, from a specimen collected 2 days later, had smooth, domed, cream colonies. Smears of both the rough and smooth cultures showed similar pleomorphic bacilli that were approximately 50% acid-alcohol-fast using Kinyoun's cold staining procedure. When 1% H₂SO₄ alone was used as the decolorizing agent, all bacilli were deeply acid-fast. Such observations are common with rapidly growing mycobacteria. No mycelia, spores, or branching were observed.

Studies of colonial morphology on 7H10 agar plates showed rudimentary filaments with no aerial hyphae or extensions. A suspension of each organism was diluted in 7H9 broth to produce isolated colonies and inoculated onto 7H10 agar, L-J, and American Trudeau Society (ATS) media. The differences between the two cultures on 7H10 medium at 2 weeks of age at 36 C are shown against contrasting backgrounds in Fig. 1. The cream colonies were considerably smaller than the brown; however, both organisms produced good growth in less than 7 days from dilute inocula.

Table 1 shows the transition of culture 1181 on the different media from buff to rust colored to brown at both 36 C and room temperature. It is interesting to note the variations in pigment

development on L-J and ATS, both egg-base media. Light had no effect on pigmentation, but there was an increase in intensity with age. The centers of the rough brown colonies took on a powdery appearance as they became older. When 7H10 plates were examined at that time under a stereoscopic microscope with reflected illumination, the colonies were observed to have irregular folds with white surface crystals.

Culture 1182 remained light cream to rosy buff, similar to *M. fortuitum*, and predominantly smooth and glistening. No brown pigment developed on any medium during 8 weeks of observation.

Differential tests (4) for speciation were performed on initial subcultures and compared with a known control strain of *M. fortuitum* (TMC 1529), type strain of the species, from the Trudeau Mycobacterial Culture Collection, Saranac Lake, N.Y. Reactions were essentially the same for both patient cultures and were similar to the properties of *M. fortuitum* with the following exceptions: a semiquantitative test for catalase of less than 45 mm; rust to brown pigment produced by culture 1181; and growth at 45 C (Table 2).

Drug susceptibility tests with both cultures gave identical results. Complete resistance was observed to the following drugs at the concentrations indicated per milliliter of 7H10 medium: streptomycin, 10 µg; *para*-aminosalicylic acid, 25 µg; isoniazid, 5 µg; ethambutol, 25 µg; rifampin, 5 µg; and ethionamide, 5 µg.

Subcultures of the rough brown and the smooth cream organisms were sent to W. B. Schaefer, Denver, Colo., for serotyping. He reported that neither agglutinated with any of the available antisera. Cultures were also sent to the VA Tuberculosis Cooperative Study Control Laboratory in Atlanta, and to the VA Spe-

FIG. 1. *Mycobacterial* cultures with identical biochemical reactions and drug susceptibility patterns, but differing in pigmentation and colonial morphology, recovered from a patient as casual isolates. One isolate was smooth and nonchromogenic; the other was rough and became dark brown. These rapidly growing organisms were most closely identified with *M. fortuitum*.

TABLE 1. Development of pigment of rough strain 1181

Temp (C)	Time of observation (days)	Medium		
		7H10	L-J	ATS
25	3	No growth	No growth	No growth
	7	Buff	Buff	Buff
	13	Buff	Buff	Buff
	15	Tan in heavy growth area; isolated colonies buff	Predominantly rust; some buff colonies	Buff
	21	Rust	Brown	Tan with scattering of brown colonies
36	3	Buff	Buff	Buff
	7	Predominantly buff; some colonies rust	Buff; starting to appear "dirty"	Buff
	13	Rust	Dark brown	Rosy buff
	15	Dark brown	Dark brown	Predominantly rosy buff; some colonies brown
	21	Dark brown	Intense brown	Tan with scattering of brown colonies

cial Reference Laboratory for Tuberculosis and Other Mycobacterial Diseases and the Mycology Research Laboratory, both at Long Beach, Calif., with requests for identification. All laboratories independently concurred with our conclusion that the organisms were not members of the genus *Nocardia* and most closely resembled the rapid grower *M. fortuitum*, based upon growth characteristics and biochemical properties (personal communications). However, the

exceptions preclude their identity with this species definitively.

Strains of *M. fortuitum* with the ability to produce pigment have been previously reported. Gordon and Pang (1) described a cream-colored *M. fortuitum* that was stored for 10 years at 4 C. Upon subculture, a black-pigmented organism grew out that had the same biochemical characteristics as the parent strain. Ross recovered strains of acid-fast bacilli

TABLE 2. Characterization of organisms

Test or property (4)	<i>M. fortuitum</i> ^a	Brown culture (1181)	Cream culture (1182)
Niacin	0	0	0
Catalase, semiquantitative, mm	>45	<45	<45
68 C/20 min	+	+	+
Tween hydrolysis, 5 days	+	+	+
Pigment production			
Light	0	+	0
Dark	0	+	0
Arylsulfatase, 3 days	+	+	+
Iron uptake	+	+	+
Nitrate reduction	+	+	+
Growth on 5% NaCl	+	+	+
Urease	+	+	+
Growth <7 days			
25 C	+	+	+
36 C	+	+	+
45 C	0	+	+
Growth on MacConkey agar	+	+	+
Colonial morphology	Smooth	Rough	Smooth

^a *M. fortuitum* (TMC 1529) used in this study was obtained from the Trudeau Mycobacterial Culture Collection sponsored by the U. S.-Japan Cooperative Medical Science Program, National Institute of Allergy and Infectious Diseases, Bethesda, Md.

similar to *M. fortuitum* from salmonoid fish that produced pale yellow pigment and a soluble purple pigment (3).

To our knowledge, the present paper is the first report of a fresh isolate from man of a culture related to *M. fortuitum* that transforms from buff to a dark brown. By its gross appearance, this pigmented organism could easily be mistaken for a fungus. A cream-colored culture, identical in all other respects, was recovered from the same patient within a 48-h period.

The tests shown in Table 2 are recommended in routine schemes for the identification of mycobacteria. These key tests have been selected

from expanded numerical analyses of data, which permit a high degree of reliability in the identification of the great majority of mycobacteria encountered in clinical specimens (2, 5). Identity is generally established on a "perfect fit" basis. If a strain deviates in a critical test or characteristic from the expected results of a given taxon, the property should be reexamined. If the same results are obtained, the culture may be referred to a specialty laboratory. Should the organism, as did cultures 1181 and 1182 in this report, again fail to conform to a definite pattern, the laboratory may report that the organism is not specifically identifiable, but, if indicated, closely resembles some known species. Although in this particular case these acid-fast bacilli, similar to *M. fortuitum*, apparently were casual isolates, microbiologists should be alert to the possibility of their occurrence in clinical specimens.

We wish to thank Milton Huppert, Irving Krasnow, and Ruth Wichelhausen for their assistance in attempting to identify these cultures. It is with deep sorrow that our expression of appreciation to Werner B. Schaefer comes posthumously.

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