Numerical Taxonomy Analysis of *Mycobacterium africanum*

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A numerical taxonomy analysis of *Mycobacterium africanum* strains from Rwanda (6 strains), Burundi (5 strains), Yaoundé (23 strains), Dakar (17 strains), and Mauretania (9 strains) indicated that these bacteria did not cluster separate from the *M. tuberculosis* or the *M. bovis* clusters, nor did they eliminate the phenetic discontinuity between the latter. However, the African strains introduced considerable heterogeneity in the usually homogeneous *M. tuberculosis* and *M. bovis* clusters. Judging from the data, it did not seem justifiable to create a new species *M. africanum*. Preliminary genetic analysis did not support the view expressed earlier that these bacteria mutated at high frequencies, and further genetic analysis of these bacteria seemed necessary.

Mycobacterium africanum Castets, Boisvert and Rist, 1969 (2) is a group of mycobacteria reported to be phenotypically intermediate between *M. bovis* and *M. tuberculosis*. The characters in common with the latter species were not constant features in strains of *M. africanum*; instead, the numerous strains investigated appeared to fill the phenetic discontinuity between *M. bovis* and *M. tuberculosis* (1, 2, 6, 7, 15-18, 20). These observations led Prat et al. (18) to suggest that the earlier classification of the tubercle bacilli, i.e., *M. tuberculosis* and its human and bovine varieties, might be reestablished and completed by newer varieties such as the African variety of tubercle bacilli. Because the distinction between *M. bovis* and *M. tuberculosis* has more than academic interest (9), we decided to reexamine the taxonomic status of *M. africanum*.

MATERIALS AND METHODS

Bacterial strains. The 98 strains of mycobacteria assembled for this investigation are listed in Table 1. Two strains each of *M. fortuitum*, *M. avium*, *M. kansasii*, *M. microti*, and *M. xenopi* were included as controls. The type or neotype strains for each of the species were included in the study.

Numerical taxonomy analysis. The tests performed on the 98 strains are indicated in Table 2. Each strain was given a code number, and the study was conducted blind. Computer analysis was carried out as recommended by Lockhart and Liston (13). Methods and strain data have been deposited with the World Data Center for Microorganisms from which they may be obtained by interested readers.

Population analysis. For the purpose of examining the genetic variation in *M. africanum*, one strain from Rwanda was arbitrarily chosen. *M. tuberculosis* and *M. bovis* were used for comparison. The fluctuation test (14) and the study of variants in the popula-

tions were performed as described previously (3, 22) and below.

RESULTS

Numerical taxonomy. The dendrogram shown in Fig. 1 was obtained excluding all strains of African origin. The strains belonging to known species, except for one strain labeled *M. microti* in our collection, separated into well defined clusters. The *M. bovis* and *M. tuberculosis* clusters separated at an 85% level of similarity. From the data, the properties of the hypothetical median strain (HMS) of *M. bovis* and *M. tuberculosis* were obtained, and all strains from these species were then compared to the HMS. The histogram (see Fig. 3A) represents the comparison of the *M. bovis* and *M. tuberculosis* strains with the *M. tuberculosis* HMS, and it indicates that the two species formed well separated and homogeneous clusters. These results indicated that the test character states selected for this investigation were satisfactory.

When the African strains were added in the analysis, we obtained the dendrogram shown in Fig. 2 and the histogram shown in Fig. 3B. The introduction of the African strains caused a 5% downward displacement of the *M. bovis*-*M. tuberculosis* intertaxon value (Fig. 1 compared with Fig. 2), and a slight overlap of heterologous organisms (Fig. 3B compared with Fig. 3A). The histogram in Fig. 3B is distinctly bimodal, and the observed displacement is not sufficient to exclude the African strains from either the *M. bovis* or the *M. tuberculosis* taxon.

The distribution of the characters with greatest resolving power between clusters F and G is shown in Table 3. The examination of Table 3 clearly shows that the distribution of characters

TABLE 1. *List of strains used in this study*

Serial no.	Lab no.	Received as:		Source ^a	Habitat	Phenon
		Name	Strain			
1	8144 A	<i>Mycobacterium fortuitum</i>	ATCC 6841	ATCC	Type strain	A
2	5194	<i>M. fortuitum</i>	HB 1792	P. Coletsos	Sputum	A
3	5911	<i>M. avium</i>	ATCC 15769	ATCC	Type strain	B
4	3897	<i>M. avium</i>	ATCC 15985	ATCC		B
5	7043	<i>M. kansasii</i>	ATCC 12478	ATCC		C
6	7959	<i>M. kansasii</i>	Hoks	H. Boisvert	Sputum	C
7	1840	<i>M. xenopi</i>	Schwabacker	P. Hauduroy	Undetermined	D
8	0068	<i>M. xenopi</i>	Lanhantec	H. Boisvert	Sputum	D
9	2627	<i>M. microti</i>	OV 168	Oxford vole	Undetermined	E
10	9910	<i>M. microti</i>	Rotterdam	Unknown	Hog	G
11	1174	<i>M. tuberculosis</i>	H 37 Rv	Trudeau Inst.	Type strain	F
12	7838	<i>M. tuberculosis</i>	HB 7838	B. Lange (1931)	Military tuberculosis (Lubeck incident)	F
13	1899	<i>M. tuberculosis</i>	Ham C-23	H. Boisvert	Pleuritis	F
14	8108	<i>M. tuberculosis</i>	Lirersky	H. Boisvert	Sputum	F
15	3337	<i>M. tuberculosis</i>	Delfosse	H. Boisvert	Sputum	F
16	6006	<i>M. tuberculosis</i>	Tanguy	H. Boisvert	Sputum	F
17	2500	<i>M. tuberculosis</i>	HB 3863	J. Grosset	Sputum	F
18	1845	<i>M. tuberculosis</i>	HB 0212	H. Boisvert	Undetermined	F
19	9731	<i>M. tuberculosis</i>	Szabo 98	I. Szabo	Undetermined	F
20	8144 C	<i>M. tuberculosis</i>	Szabo 89	A. Tacquet	Undetermined	F
21	0164	<i>M. bovis</i>	ATCC 19210	ATCC	Type strain	G
22	9222	<i>M. bovis</i>	HB 1844	G. Canetti	Sputum	G
23	2062	<i>M. bovis</i>	HB 2582	Vallée	Cat	G
24	1905	<i>M. bovis</i>	HB 3439	CDC	Monkey	G
25	3142	<i>M. bovis</i>	HB 6966	H. Boisvert	Undetermined	G
26	8372	<i>M. bovis</i>	HB 3313	H. Boisvert	Undetermined	G
27	5374	<i>M. bovis</i>	Barthelemy	H. Boisvert	Meningitis	G
28	4043	<i>M. bovis</i>	HB 3882	H. Boisvert	Undetermined	G
29	0517	<i>M. bovis</i>	HB 3625	H. Boisvert	Sputum	G
30	7553	<i>M. bovis</i>	HB 3513	H. Boisvert	Sputum	G
31	3393	<i>M. bovis</i>	HB 3889	H. Boisvert	Undetermined	G
32	2680	<i>M. bovis</i>	HB 6712	H. Boisvert	Undetermined	G
33	4298	<i>M. bovis</i>	HB 1849	H. Boisvert	Undetermined	G
34	6271	<i>M. bovis</i>	HB 1573	H. Boisvert	Undetermined	G
35	8638	<i>M. bovis</i>	HB 1864	H. Boisvert	Undetermined	G
36	1736	<i>M. bovis</i>	Neveu	H. Boisvert	Cow	G
37	7791	<i>M. bovis</i>	HB 7199	H. Boisvert	Undetermined	G
38	9380	<i>M. bovis</i>	HB 2571	P. Vergez	Sputum	G
39	8916	<i>M. africanum</i>	RT 6833	Rwanda	Sputum	F
40	2596	<i>M. africanum</i>	RT 6856	Rwanda	Sputum	F
41	6749	<i>M. africanum</i>	RT 6854	Rwanda	Sputum	F
42	2617	<i>M. africanum</i>	RT 6832	Rwanda	Sputum	F
43	6204	<i>M. africanum</i>	RT 6862	Rwanda	Sputum	F
44	0131	<i>M. africanum</i>	RT 8834	Burundi	Sputum	F
45	8726	<i>M. africanum</i>	RT 8829	Burundi	Sputum	F
46	2523	<i>M. africanum</i>	RT 8818	Burundi	Sputum	F
47	0010	<i>M. africanum</i>	RT 8815	Burundi	Sputum	F
48	0063	<i>M. africanum</i>	RT 8811	Burundi	Sputum	F
49	2315	<i>M. africanum</i>	RT 5901	Yaoundé	Sputum	G
50	0554	<i>M. africanum</i>	RT 6619	Yaoundé	Sputum	G
51	1487	<i>M. africanum</i>	RT 6615	Yaoundé	Sputum	G
52	4336	<i>M. africanum</i>	RT 6899	Yaoundé	Sputum	G
53	4954	<i>M. africanum</i>	RT 6465	Yaoundé	Sputum	G
54	0709	<i>M. africanum</i>	RT 6464	Yaoundé	Sputum	G
55	4331	<i>M. africanum</i>	RT 6378	Yaoundé	Sputum	F
56	6157	<i>M. africanum</i>	RT 6467	Yaoundé	Sputum	G
57	3135	<i>M. africanum</i>	RT 6470	Yaoundé	Sputum	G
58	5704	<i>M. africanum</i>	RT 5897	Yaoundé	Sputum	G

TABLE 1.—Continued

Serial no.	Lab no.	Received as:		Source ^a	Habitat	Phenon
		Name	Strain			
59	0924	<i>M. africanum</i>	RT 611	Yaoundé	Sputum	G
60	9795	<i>M. africanum</i>	RT 6003	Yaoundé	Sputum	G
61	5350	<i>M. africanum</i>	RT 6114	Yaoundé	Sputum	G
62	1112	<i>M. africanum</i>	RT 6010	Yaoundé	Sputum	G
63	5901	<i>M. africanum</i>	RT 6002	Yaoundé	Sputum	G
64	4310	<i>M. africanum</i>	RT 6737	Yaoundé	Sputum	G
65	5032	<i>M. africanum</i>	RT 5895	Yaoundé	Sputum	G
66	9224	<i>M. africanum</i>	RT 6014	Yaoundé	Sputum	G
67	7433	<i>M. africanum</i>	RT 6137	Yaoundé	Sputum	G
68	8717	<i>M. africanum</i>	HB 3657	Yaoundé	Sputum	G
69	0495	<i>M. africanum</i>	HB 3656	Yaoundé	Sputum	G
70	3623	<i>M. africanum</i>	HB 3807	Yaoundé	Sputum	G
71	2837	<i>M. africanum</i>	RT 5900	Yaoundé	Sputum	G
72	2329	<i>M. africanum</i>	HB 5222	Yaoundé	Sputum	G
73	7210	<i>M. africanum</i>	ATCC 25420	Dakar	Sputum	G
74	8948	<i>M. africanum</i>	HB 1702	Dakar	Sputum	G
75	7819	<i>M. africanum</i>	HB 2048	Dakar	Sputum	G
76	9226	<i>M. africanum</i>	HB 2827	Dakar	Sputum	G
77	3712	<i>M. africanum</i>	HB 1659	Dakar	Sputum	G
78	0559	<i>M. africanum</i>	HB 1699	Dakar	Sputum	G
79	8144 B	<i>M. africanum</i>	RT 8458	Dakar	Sputum	G
80	8865	<i>M. africanum</i>	RT 6088	Dakar	Sputum	G
81	3442	<i>M. africanum</i>	RT 5273	Dakar	Sputum	G
82	2595	<i>M. africanum</i>	RT 8455	Dakar	Sputum	G
83	0744	<i>M. africanum</i>	RT 8456	Dakar	Sputum	G
84	9854	<i>M. africanum</i>	RT 6807	Dakar	Sputum	G
85	0971	<i>M. africanum</i>	RT 6091	Dakar	Sputum	G
86	6882	<i>M. africanum</i>	RT 8454	Dakar	Sputum	G
87	3317	<i>M. africanum</i>	RT 5272	Dakar	Sputum	G
88	2549	<i>M. africanum</i>	RT 8432	Dakar	Sputum	G
89	1288	<i>M. africanum</i>	RT 8982	Mauretania	Sputum	G
90	9373	<i>M. africanum</i>	RT 8945	Mauretania	Sputum	G
91	7262	<i>M. africanum</i>	RT 8967	Mauretania	Sputum	G
92	6102	<i>M. africanum</i>	RT 8946	Mauretania	Sputum	G
93	9783	<i>M. africanum</i>	RT 8935	Mauretania	Sputum	G
94	1132	<i>M. africanum</i>	RT 8966	Mauretania	Sputum	G
95	7548	<i>M. africanum</i>	RT 8970	Mauretania	Sputum	G
96	5550	<i>M. africanum</i>	RT 8974	Mauretania	Sputum	G
97	1603	<i>M. africanum</i>	RT 8985	Mauretania	Sputum	G
98	2693	<i>M. africanum</i>	RT 8980	Mauretania	Sputum	G

^a ATCC, American Type Culture Collection, Rockville, Md.; CDC, Center for Disease Control, Atlanta, Ga.

in clusters F and G from dendrogram 1 is homogeneous, whereas the distribution of characters in the same clusters from dendrogram 2 is rather heterogeneous. Therefore, although the African strains did not eliminate the phenetic discontinuity that justifies the separation of the species *M. bovis* from *M. tuberculosis*, they introduced considerable heterogeneity in both taxa.

Subclusters in cluster F (from dendrogram 2). Subcluster F₁ was composed by one strain from Szabö ("green" strains) and one strain from Boisvert that was identified by him before as of the Szabö type. Subcluster F₂ con-

tained three strains from Rwanda, two from Burundi, and one from Yaoundé. Subcluster F₃ contained seven strains of *M. tuberculosis* including the neotype strain ATCC 27294, one strain from Rwanda, and two strains from Burundi.

Subclusters in cluster G (from dendrogram 2). Subcluster G₁ contained four strains from Yaoundé, two from Dakar, and one from Mauretania. Subcluster G₂ contained fourteen strains from Yaoundé, four strains from Mauretania, two strains from Dakar, one strain from Rwanda, and one strain of *M. bovis*. Subcluster G₃ contained four strains from Yaoundé, thir-

teen strains from Dakar, four strains from Mauritania, one strain labeled *M. microti* Rotterdam, and seventeen strains of *M. bovis*. The neotype strain *M. bovis* ATCC 19219 and the type strain *M. africanum* ATCC 25420 were included in this last subcluster.

Comparison of the HMS with the type or neotype strains of *M. bovis*, *M. tuberculosis*, and *M. africanum*. The comparison of the characters of the neotype or type strains with the characters of the HMS strains of *M. tuber-*

culosis and *M. bovis* (Table 4) showed that there was complete agreement between *M. tuberculosis* ATCC 27294 and *M. tuberculosis* HMS. *M. bovis* ATCC 19210 differed from *M. bovis* HMS in two characters, and *M. africanum* ATCC 25420 differed from the *M. bovis* HMS strain in three characters.

Geographic distribution. The distribution of the African strains that clustered in the *M. bovis* taxon and in the *M. tuberculosis* taxon in respect to their geographic origin is depicted in

TABLE 2. Properties exhibited by *M. bovis*, *M. tuberculosis*, and the strains in clusters F and G from the dendrogram shown in Fig. 2

Character	<i>M. tuberculosis</i>	<i>M. bovis</i>	Clusters	
			F	G
Bacilli (2 μ m long)	+ (100)	+ (100)	+ (100)	+ (100)
Coccobacilli (2 μ m long)	0 (0)	0 (0)	0 (0)	0 (0)
Cording	+ (71)	0 (0)	0 (43)	0 (46)
Growth at 37°C:				
7 days or less	0 (0)	0 (0)	0 (0)	0 (0)
Greater than 7 days	+ (100)	+ (100)	+ (100)	+ (100)
Colony type: ^a				
Smooth S, T, D or I	0 (0)	0 (0)	0 (0)	0 (0)
Rough R	+ (52)	+ (82)	0 (41)	0 (38)
Rough R _s	0 (29)	0 (47)	0 (15)	0 (29)
Rough R _w	0 (43)	0 (6)	0 (24)	0 (10)
Rough Robb	0 (29)	0 (0)	0 (21)	0 (23)
Lebek niveau ^b	0 (10)	+ (88)	0 (26)	+ (87)
Pigment in dark ^c	0 (0)	0 (0)	0 (0)	0 (0)
Pigment in light ^c	0 (0)	0 (0)	0 (0)	0 (0)
Growth temperature				
20°C	0 (0)	0 (0)	0 (0)	0 (0)
30°C ^d	+ (100)	+ (100)	+ (100)	+ (100)
37°C	+ (100)	+ (100)	+ (100)	+ (100)
42°C	0 (0)	0 (0)	0 (0)	0 (0)
52°C	0 (0)	0 (0)	0 (0)	0 (0)
Growth stimulation by pyruvate ^e	0 (0)	+ (100)	0 (48)	+ (100)
Growth on nutrient agar	0 (0)	0 (0)	0 (0)	0 (0)
Acid from fructose	0 (0)	0 (0)	0 (0)	0 (0)
5% NaCl tolerance	0 (0)	0 (0)	0 (0)	0 (0)
Nitrite inhibition (25 mM) ^f	+ (100)	+ (100)	+ (100)	+ (100)
Niacin ^g	+ (100)	0 (0)	+ (86)	0 (21)
Catalase: ^c				
40 mm or less bubbles	+ (100)	+ (100)	+ (100)	+ (100)
40 mm or more bubbles	0 (0)	0 (0)	0 (0)	0 (4)
After heating at 68°C	0 (0)	0 (0)	0 (0)	0 (0)
Peroxidase: ^h				
Room temp	+ (100)	+ (100)	+ (100)	+ (100)
After heating at 68°C	0 (0)	0 (0)	0 (0)	0 (0)
Nitrate red (Tacquet) ⁱ	+ (100)	0 (0)	+ (100)	0 (30)
Nitrate red (Virtanen) ^c	+ (100)	0 (0)	+ (81)	0 (4)
Nitrite red (Tacquet) ⁱ	0 (14)	0 (0)	0 (5)	0 (0)
Acid phosphatase ^j	+ (100)	+ (94)	+ (81)	+ (66)
Alkaline phosphatase ^k	+ (100)	+ (94)	+ (62)	0 (43)
Tween hydrolysis ^l	0 (14)	0 (0)	0 (5)	0 (3)
Urease ^m	+ (100)	+ (76)	+ (95)	0 (26)
Arylsulfatase ^c	0 (0)	0 (0)	0 (0)	0 (0)
β -Glucosidase ⁿ	+ (100)	0 (0)	0 (43)	0 (1)
Pyrazinamidase ^o	+ (100)	0 (0)	+ (95)	0 (6)

TABLE 2.—Continued

Character	<i>M. tuberculosis</i>	<i>M. bovis</i>	Clusters	
			F	G
Growth in presence of: ^p				
INH (0.1 µg/ml)	0 (0)	0 (0)	0 (0)	0 (0)
TCH (2.0 µg/ml)	+ (100)	0 (0)	+ (81)	0 (0)
PZA (400.0 µg/ml)	0 (29)	+ (88)	0 (14)	0 (24)
Tb ₁ (2.0 µg/ml)	0 (0)	0 (0)	0 (0)	0 (31)
PAS (0.5 µg/ml)	0 (0)	0 (7)	0 (0)	0 (4)
SM (4.0 µg/ml)	0 (0)	0 (0)	0 (0)	0 (1)
EMB (2.0 µg/ml)	0 (0)	0 (0)	0 (0)	0 (0)
D-CS (30.0 µg/ml)	0 (0)	0 (0)	0 (0)	0 (0)
RIF (40.0 µg/ml)	0 (0)	0 (0)	0 (0)	0 (0)
KAN (20.0 µg/ml)	0 (0)	0 (0)	0 (0)	0 (0)
VM (30.0 µg/ml)	0 (0)	0 (0)	0 (0)	0 (0)
CAP (40.0 µg/ml)	0 (0)	0 (0)	0 (0)	0 (0)
ETH (20.0 µg/ml)	0 (0)	0 (0)	0 (0)	0 (8)

^a All strains gave rough colonies. Colony types S, T, D, and I are as described by Vestal (21). Abbreviations: rough R, rough with coarse cords; R_m, rough with central button; R_w, rough, dark, and compact; and Robb, rough, opaque, and with folded edges.

^b Lebek (12).

^c Vestal (21).

^d All strains grew at 30°C, but growth was poor.

^e Castets et al. (1).

^f Kestle, Abbott, and Kubica (10).

^g Niacin test strip (Difco).

^h Boisvert, personal communication.

ⁱ Tacquet and Tison (19).

^j Kapler (8).

^k David (4).

^l Tween hydrolysis substrate (Difco).

^m Urea-indole, Pasteur. About 10.0 mg of cells was suspended in 1.0 ml of the substrate. The mixture was incubated overnight at 37°C; development of a red color indicated a positive reaction.

ⁿ David and Jahan (5).

^o About 10.0 mg of cells was suspended in 1.0 ml of a 10⁻³ M solution of pyrazinamide in tris(hydroxymethyl)aminomethane buffer (Sigma), pH 7.2. The mixture was incubated overnight at 37°C, when the presence of pyrazonoic acid was determined by the addition of crystals of ferrous ammonium sulfate.

^p In Lowenstein-Jensen medium. Abbreviations: INH, isoniazid; TCH, thiophencarboxylic acid hydrazide; PZA, pyrazinamide; Tb₁, amithiazone; PAS, *p*-amino salicylic acid; SM, streptomycin; EMB, ethambutol; D-CS, D-cycloserine; RIF, rifampin; KAN, kanamycin; VM, viomycin; CAP, capreomycin; ETH, ethionamide.

TABLE 3. Distribution of characters with greatest resolving power between *M. bovis* and *M. tuberculosis*, between clusters G and F from Fig. 2, and within clusters G and F

Test	<i>M. bovis</i>	<i>M. tuber- culosis</i>	Cluster		Subcluster					
			F	G	G ₁	G ₂	G ₃	F ₁	F ₂	F ₃
Lebek niveau	100	0	87	26	83	71	90	0	29	22
Growth stimulation by pyruvate	100	0	100	48	100	100	100	0	71	30
Niacin	0	100	21	86	100	50	16	100	71	70
Nitrate red (Tacquet)	0	100	30	100	100	100	24	100	100	100
Nitrate red (Virtanen)	0	100	4	81	100	0	16	100	57	100
Acid phosphatase	94	100	66	81	71	68	64	100	50	100
Alkaline phosphatase	94	100	43	62	43	41	44	100	0	90
Urease	76	100	26	95	71	18	38	100	100	100
β-Glucosidase	0	100	1	43	100	0	0	100	0	60
Pyrazinamidase	0	100	6	95	0	0	7	100	100	100
Growth in presence of: ^a										
TCH (2.0 μg/ml)	0	100	0	81	0	0	0	100	86	100
PZA (400.0 μg/ml)	88	29	24	14	0	0	27	33	0	20
Tb ₁ (2.0 μg/ml)	0	0	31	0	71	50	13	0	0	0

^a Abbreviations: TCH, thiophencarboxylic acid hydrazide; PZA, pyrazinamide; Tb₁, amithiazone.

FIG. 1. Dendrogram of 38 mycobacteria selected with intended bias to assess the taxonomic value of the chosen tests. Cluster A, *M. fortuitum*; cluster B, *M. avium*; cluster C, *M. kansasii*; cluster D, *M. xenopi*; cluster E, *M. microti*; cluster F, *M. tuberculosis*; and cluster G, *M. bovis*. Strain 1, *M. fortuitum* ATCC 1792; strain 2, *M. avium* ATCC 15769; strain 3, *M. kansasii* ATCC 12478; strain 4, *M. xenopi* ATCC 19276; strain 5, *M. microti*, Oxford vole 168; strain 6, *M. tuberculosis* ATCC 27294; and strain 7, *M. bovis* ATCC 19210.

FIG. 2. Dendrogram of 89 strains of *M. tuberculosis*, *M. bovis*, and *M. africanum*. Cluster F₁, *M. tuberculosis*; cluster G₁, *M. bovis*. In addition to *M. tuberculosis*, cluster F contained 6 strains from Rwanda, 5 strains from Burundi, and 1 strain from Yaoundé; in addition to *M. bovis*, cluster G contained 22 strains from Yaoundé, 17 strains from Dakar, 9 strains from Mauretania, and 1 strain labeled *M. microti* Rotterdam. For the distribution of the strains in the recognizable subclusters, see Table 5. Strain 1, *M. tuberculosis* ATCC 27294; strain 2, *M. bovis* ATCC 19210; strain 3, *M. africanum* ATCC 25420.

FIG. 3. Histograms obtained by comparing all strains in clusters F and G with the hypothetical medium strain of *M. tuberculosis*. (A) Strains in clusters F and G from dendrogram in Fig. 1; (B) strains in clusters F and G from dendrogram in Fig. 2.

Table 5. All strains except one from West Africa (Yaoundé, Dakar, and Mauretania) were in the *M. bovis* cluster G, and all strains except one from Rwanda and Burundi were in the *M. tuberculosis* cluster F.

Genetic variation. The phenetic analysis described above excluded *M. africanum* as a distinct species, and suggested that the African strains are variants of either *M. bovis* or *M. tuberculosis*. It was reported earlier (18) that the African strains mutate at high frequencies which could explain the heterogeneity introduced by these strains in the *M. bovis* and *M. tuberculosis* clusters. The mutation rates of a strain from Rwanda from sensitivity towards resistance to isoniazid, streptomycin, rifampin, and kanamycin did not differ significantly from the correspondent mutation rates in *M. bovis* and *M. tuberculosis* (Table 6). On the other hand, these bacteria exhibited considerable variation in results in the various tests shown in Table 7. These data, therefore, excluded mutator effects in the strain but indicated a genetic phenomenon that requires further examination.

DISCUSSION

Our data showed conclusively that the African strains of tubercle bacilli did not segregate into a cluster separate from *M. bovis* or *M. tuberculosis*, leading us to question the validity of the species *M. africanum* Castets, Boisvert and Rist, 1969. Furthermore, our data did not lend support to the proposal that the African strains are intermediate organisms linking *M. bovis* and *M. tuberculosis* in a single species and several varieties (18). The African strains clustered with either *M. bovis* or *M. tuberculosis*, and although they introduced in *M. tuberculosis* considerable

TABLE 4. Reactions of the HMS of *M. tuberculosis* and the neotype strain ATCC 27294 (11), and the HMS of *M. bovis*, the neotype strain ATCC 19210 (9), and the type strain ATCC 25420 of *M. africanum* (2)

Test	<i>M. tuberculosis</i>		<i>M. bovis</i>		
	HMS	ATCC 27294	HMS	ATCC 19210	ATCC 25420
Lebek niveau	0	0	+	+	+
Growth stimulation by pyruvate	0	0	+	+	+
Niacin	+	+	0	0	0
Nitrate reductase (Tacquet)	+	+	0	0	0
Nitrate reductase (Virtanen)	+	+	0	0	0
Acid phosphatase	+	+	+	+	+
Alkaline phosphatase	+	+	+	0	0
Urease	+	+	+	0	0
β -Glucosidase	+	+	0	0	0
Pyrazinamidase	+	+	0	0	0
Growth in presence of: ^a					
TCH (2.0 μ g/ml)	+	+	0	0	0
PZA (400.0 μ g/ml)	0	0	+	+	0
Tb ₁ (2.0 μ g/ml)	0	0	0	0	0

^a Abbreviations: TCH, thiophencarboxylic acid hydrazide; PZA, pyrazinamide; Tb₁, amithiazone.

heterogeneity, the phenetic discontinuity between the two taxa was not eliminated. It may be argued, however, that the observed (albeit slight) downward displacement in the intertaxon value might have been more apparent, or per-

haps would have disappeared, had we used a much larger number of strains in our study. In this respect it was interesting that the African strains clustered according to their geographic origin. Most strains from West Africa clustered in the *M. bovis* cluster G, and the strains received from the more eastward region of Africa (Rwanda and Burundi) were found in the *M. tuberculosis* cluster F. Strains received from regions between these two extremes were not available for study.

Even if the study of many more strains might cause the clusters to fuse, the data implied a genetic diversity that requires clarification. The careful and detailed descriptions by Rist and his collaborators (1, 2, 7, 18) led them to suspect that these bacteria mutated at high frequencies. We tested this hypothesis by measuring the

TABLE 5. *Distribution of the African strains in the M. bovis taxon and in the M. tuberculosis taxon, in respect to their geographic origin*

Geographic origin	<i>M. bovis</i> taxon (cluster G)			<i>M. tuberculosis</i> taxon (cluster F)		
	G ₁	G ₂	G ₃	F ₁	F ₂	F ₃
Rwanda	0	1	0	0	3	1
Burundi	0	0	0	0	2	2
Yaoundé	4	14	4	0	1	0
Dakar	2	1	13	0	0	0
Mauretania	1	4	4	0	0	0

TABLE 6. *Mutation rates (α) from susceptibility towards resistance to the indicated drugs of M. tuberculosis, M. bovis, and a strain (RT 6858) from Rwanda*

Drug ^a	<i>M. tuberculosis</i>		<i>M. bovis</i>		Rwanda RT 6858	
	α	Mf ^b	α	Mf	α	Mf
INH	9×10^{-8}	6.92	1.3×10^{-8}	1.0	1.6×10^{-7}	12.3
STM	7.1×10^{-9}	1.39	5.1×10^{-9}	1.0	1.6×10^{-8}	3.1
RIF	3.3×10^{-8}	1.32	2.5×10^{-8}	1.0	7.5×10^{-8}	3.0
KAN	9.1×10^{-9}	0.95	9.6×10^{-9}	1.0	4.2×10^{-8}	4.4

^a The concentrations of isoniazid (INH), streptomycin (STM), rifampin (RIF), and kanamycin (KAN) were, respectively, 1.0 μ g/ml, 2.0 μ g/ml, 1.0 μ g/ml, and 5.0 μ g/ml.

^b Mf, Mutation factor = (mutation rate of test organism)/(mutation rate of reference). In these calculations, *M. bovis* was used as the reference organism.

TABLE 7. *Phenotypes of clones, and subcultures, of strain RT 6858 of M. africanum (Rwanda)*

Test condition ^a	Pyruvate stimulation	Nitrate reductase		β -Glucosidase	Niacin	Pyrazinamidase	Growth in the presence of TCH ^b
		Tacquet	Virtanen				
A ¹	40	100	45	0	100	45	99
A ²	61	100	69	7	92	46	100
A ³	20	100	80	0	60	0	100
A ⁴	38	100	69	0	100	23	100
B	1		+	0	+	0	
	2		0	0	+	+	
	3		+	0	+	+	
	4		0	0	+	+	
	5		+	0	+	0	
	6		0	0	+	0	
C	0	+	0	0	+	+	+

^a Test condition A: Percent of positive clones in the indicated tests. A¹ = 12 colonies cloned from culture no. 3 in the fluctuation test; A² = 13 colonies cloned from culture no. 16 in the fluctuation test; A³ = 10 colonies cloned from isoniazid-containing plates in the fluctuation test; A⁴ = 13 colonies cloned from kanamycin-containing plates in the fluctuation test. Test condition B: Growth on the surface and in the depth of Lebeck medium was carefully transferred to Lowenstein-Jensen slants. The cultures were allowed to grow, and the indicated tests were then performed as usual. (1) Parental strain, growth on the surface. (2) Parental strain, growth in depth. (3) Eugonic clone from RT 6858, growth on the surface. (4) Eugonic clone from RT 6858, growth in depth. (5) Dysgonic clone from RT 6858, growth on the surface. (6) Dysgonic clone from RT 6858, growth in depth. Test condition C: The parental strain RT 6858 was tested as usual.

mutation rates towards resistance to drugs of a strain from Rwanda, and did not find evidence for mutator effects. Yet, the population analysis in respect to test results in biochemical tests showed variations at a frequency that cannot be explained by spontaneous mutations. Therefore, we think that the fundamental mechanism of the genetic instability of these organisms needs to be further investigated. Because we have observed that the genetic instability was found for some but not all of the test characters, we hypothesize that it may be caused by extrachromosomal genes. This hypothesis is now under investigation.

From a practical point of view, the differential identification of *M. bovis* and *M. tuberculosis*, where and when African strains must be identified, is complicated by the need to perform more laboratory tests than is usually recommended. According to our data, the minimal tests necessary to identify conclusively the two species are growth stimulation by pyruvate, niacin production, nitrate reductase, β -glucosidase, pyrazinamidase, and growth in the presence of thiophencarboxylic acid hydrazide. In our opinion strains that do not give the expected test results in one or more of the indicated tests must be further investigated.

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REPRINT REQUESTS

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