Mycobacterium malmøense sp. nov.

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Strains of a new type of group III nonphotochromogenic mycobacteria have been repeatedly isolated from four patients with clinical and roentgenological signs of lung mycobacteriosis. These strains split nicotinamide and pyrazinamide as do those of *Mycobacterium avium*, but they show no esterase activity. Furthermore, the strains produce thermolabile catalase and hydrolyze Tween 80. They also have a unique lipid pattern and special sensitins and agglutinins. These strains, therefore, are considered as belonging to a new species of pathogenic, nonphotochromogenic mycobacteria, for which we propose the name *Mycobacterium malmøense*. Strain M-ö 816 is the type strain, and it has been deposited in the American Type Culture Collection under the number 29571.

During the years 1968 through 1970, seven unusual mycobacterial strains were isolated from four patients from Malmö and Lund, Sweden. All of the strains grew extremely slowly and poorly as tiny colonies. Their properties, as determined at laboratories in Malmö, Sweden, and in Borstel, Germany, indicated that they belonged to a new species. This paper reports the results of a taxonomic study of these strains.

MATERIALS AND METHODS

Bacterial strains. The seven strains of atypical mycobacteria studied (referred to herein as the Malmö strains) and their sources are cited below. Strain M-ö 648 was isolated from a needle aspiration of a process in the right lung, and strains M-ö 649 and M-ö 650 were isolated from gastric lavage and bronchial secretion, respectively, of the patient. Strains M-ö 709 and M-ö 710 were isolated from gastric lavage and sputum, respectively, from the second patient, who had two more sputum samples which were culture positive. Strain M-ö 716 was isolated from the sputum of the third patient, who had four more positive samples. Strain M-ö 816 was isolated from resected lung tissue in the fourth patient, who also had positive sputum cultures.

The results of earlier studies (11) of strains of *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium xenopi* were compared with the properties of these new strains.

Strain characteristics. Colony morphology and the ability of the strains to grow at various temperatures (22, 31, 37, 39, 43, and 45°C) were observed during 6 weeks of incubation of cultures on Löwenstein-Jensen medium inoculated with 10^{-3} mg of mycobacteria per tube.

Drug susceptibility testing was performed with Löwenstein-Jensen medium in tubes closed with stoppers which allowed exchange of air. Chemotherapeutic agents were added before inspissation. Tubes containing isoniazid, streptomycin, ethambu-

tol, rifampin, *p*-aminosalicylic acid, or thiosemicarbazone were inoculated with 10^{-3} mg of mycobacteria per tube; those with ethionamide, capreomycin, viomycin, kanamycin, or cycloserine were inoculated with 10^{-4} mg per tube. Two control tubes were included in each test series, as were two additional controls, with an inoculum of 10^{-5} mg of bacteria per tube. Cultures were incubated for 4 weeks at 37°C. Strains were reported as resistant if they grew in the tubes containing the critical concentrations of agent used (see Table 1) and showed at least 1% (inoculum, 10^{-3} mg of mycobacteria per ml) or 10% (inoculum, 10^{-4} mg of mycobacteria per ml) of the growth appearing in the control tubes; they were reported as susceptible if they did not grow at these concentrations. The range between no growth and growth of 1% of that in the control tubes is called "doubtful."

The influence of oxygen on growth was investigated in the oxygen gradient of a semisolid agar column (Lebek test, modification from Schmiedel and Gerloff [14]), and the production of acid from glycerol was determined in egg medium with bromocresol purple as an indicator (6, 14).

Other properties studied included amidase pattern (1), production of nicotinic acid (12), reduction of nitrate (2), hydrolysis of Tween 80 (16), production of phosphatase (4), esterases (5), arylsulfatase (9), and catalase, and the thermolability of catalase (3).

For pathogenicity tests (10), 4-week-old cultures were used. Hens and rabbits were infected intravenously and guinea pigs were infected subcutaneously with 1 ml of a mycobacterial suspension of 10^{-2} mg of mycobacteria per ml of each strain. After 8 weeks, the animals were sacrificed if they had not already died. Reisolations were made from liver, spleen, and lymph nodes.

Serological studies were performed by W. Anz and G. Meissner (Borstel, Germany) and by W. B. Schaefer (Denver, Colo.) by the method described by Schaefer (13), lipid patterns were analyzed by P. Jenkins (Cardiff, England) (8), and an investigation of sensitins was made by M. Magnusson (Copenhagen, Denmark) (7).

RESULTS

Microscopic observation of cells of the seven strains grown on Löwenstein-Jensen medium revealed coccoid to short rods, but, when grown on Middlebrook and Cohn 7H10 agar or cornmeal agar, the cells were short to moderately long rods, acid alcohol-fast, and gram positive but without such characteristics as cords, cross-bands, or interwoven networks. No spores, capsules, or aerial hyphae could be seen.

On Löwenstein-Jensen medium, all of the strains grew as dysgonic, smooth, colorless colonies when incubated from 22 to 37°C. Growth at 22°C was observed only after 6 weeks of incubation. The colonies remained colorless after exposure to light. Dilute inocula on Middlebrook and Cohn 7H10 agar yielded smooth, glistening, grayish-white, opaque, domed, circular colonies, 0.5 to 1.5 mm in diameter with entire margins, and some umbonate colonies, 0.6 to 2.7 mm in diameter, with compact, raised centers and flattened, irregular edges (Fig. 1). On cornmeal agar, the colonies were smaller, 0.6 to 1.7 mm in diameter, grayish-white, um-

bonate with raised, compact centers surrounded by thin granular borders with entire or slightly irregular edges; some low, convex, circular, coarsely granular colonies, 0.9 to 1.7 mm in diameter, with sharp, entire edges were also produced (Fig. 2 and 3). Although the strains have been subcultivated over a period of several years, growth is still very slight upon subculturing.

As shown in Table 1, the strains were susceptible to 1 µg of ethambutol, 16 µg of ethionamide, 8 µg of kanamycin, and 16 µg of cycloserine and were resistant to the remaining antibacterial agents used. The susceptibilities of the strains to viomycin were not uniform.

In the oxygen gradient of the Lebek test, the Malmö strains grew at the same depth as *Mycobacterium bovis*, which means that they needed less oxygen than other mycobacteria. As with *M. bovis*, they grew without color change in the bromocresol purple medium.

None of the strains produced nicotinic acid, and none of them reduced nitrate; however, all of them split the amides nicotinamide and pyrazinamide. Tween 80 was hydrolyzed, and all of

FIG. 1. A smooth, glistening colony (right) and an umbonate colony (left) with a raised compact center and with flattening at the periphery. Middlebrook and Cohn 7H10 agar. Strain M-ö 816.

FIG. 2. A smooth, glistening colony (right) and an umbonate colony (left) with a raised, compact center and with flattening at the periphery. Cornmeal agar. Strain M-ö 816.

the strains produced catalase, which was inactivated by heating at 68°C. Phosphatase and α - and β -esterases were not detectable, and the arylsulfatase test was negative. In Table 2, some of the properties of the Malmö strains are compared with those of closely related mycobacteria.

In guinea pigs, all of the strains produced a strong local reaction but no generalization. In hens, four of the seven strains caused macroscopic lesions in the liver and spleen, and two of the hens died before the end of the test. A heavy growth of mycobacteria appeared when cultures were made from the organs of these hens; no growth was obtained from specimens from the surviving hens. The rabbits showed few or no lesions; specimens from liver and spleen gave either no growth or only single colonies.

The serological investigation of the agglutinins (performed by W. Anz and G. Meissner, Borstel, Germany) revealed that all of the strains are of the same serotype, one which

differs from those previously known. A description of this new serotype will be published later. Furthermore, all of the strains showed the same unique lipid pattern (determined by P. A. Jenkins, Cardiff, England). Their sensitivities were similar, too, and they deviated from those of *M. avium*, *M. intracellulare*, *M. gastri*, *M. kansasii*, and *M. tuberculosis* (with which they were compared (by M. Magnusson, Copenhagen, Denmark)).

DISCUSSION

The Malmö strains are similar to those of *M. avium*, *M. intracellulare*, and *M. xenopi* with regard to hen pathogenicity, amidase pattern, and inability to reduce nitrate. They are differentiated from strains of the *M. avium* complex by the phosphatase-esterase pattern, which is specific for *M. avium* and related species, and by the thermolabile catalase. The ability to hydrolyze Tween 80 is an unusual property for pathogenic strains because this property ordi-

FIG. 3. A small, smooth, glistening colony (lower left) and several umbonate colonies with compact centers and flattened peripheries. Cornmeal agar. Strain M-ö 816.

TABLE 1. Susceptibilities of the Malmö strains and related mycobacteria to antimicrobial agents^a

Taxa	Isonia- zid (0.25 µg/ml)	Strepto- mycin (4.0 µg/ ml)	Etham- butol (1.0 µg/ml)	Rifam- pin (32.0 µg/ml)	p-Amino- salicylic acid (0.5 µg/ml)	Thiosem- icarba- zone (1.0 µg/ml)	Ethion- amide (16.0 µg/ml)	Viomy- cin (16.0 µg/ml)	Kana- mycin (8.0 µg/ml)	Ca- preo- mycin (16.0 µg/ml)	Cyclo- serine (16.0 µg/ml)
Strains:											
M-ö 648	R	R	S	R	R	R	S	R	S	R	S
M-ö 649	R	R	S	R	R	R	S	S	S	R	S
M-ö 650	R	R	S	R	R	R	S	R	S	R	S
M-ö 709	R	R	S	R	R	R	S	S	S	R	S
M-ö 710	R	R	S	R	R	R	S	R	S	R	S
M-ö 716	R	R	S	R	R	R	S	S	S	R	S
M-ö 816	R	R	S	R	R	R	S	S	S	R	S
Species:											
<i>M. avium</i>	R	R	R	R	R	R	Var	R	R	R	S
<i>M. intra- cellu- lare</i>	R	R	R	R	R	R	S	R	R	R	S
<i>M. xenopi</i>	S ^x	S	S ^x	S	R	R	S	S	S	S	S

^a Symbols: R, resistant; S, susceptible; S^x, susceptible, but resistance may occur; Var, results not uniform.

TABLE 2. Selected properties of the Malmö strains in comparison with those of closely related mycobacteria^a

Taxa	Amidase activity ^b						Nitrate reduc- tase	Phospha- tase	Esterase		Catalase		Hydrolysis of Tween 80	Arylsul- fatase
	1	2	3	5	6	9			α	β	22°C	68°C		
Strains:														
M-ö 648	0	0	0	+	+	0	0	0	0	0	+	0	+	0
M-ö 649	0	0	0	+	+	0	0	0	0	0	+	0	+	0
M-ö 650	0	0	0	+	+	0	0	0	0	0	+	0	+	0
M-ö 709	0	0	0	+	+	0	0	0	0	0	+	0	+	0
M-ö 710	0	0	0	+	+	0	0	0	0	0	+	0	+	0
M-ö 716	0	0	0	+	+	0	0	0	0	0	+	0	+	0
M-ö 816	0	0	0	+	+	0	0	0	0	0	+	0	+	0
Species:														
<i>M. avium</i>	0	0	0	+	+	0	0	0	+	+	+	+	0	0
<i>M. intracellu- lare</i>	0	0	0	+	+	0	0	0	+	+	+	+	0	0
<i>M. xenopi</i>	0	0	0	+	+	0	0	0	+	+	+	+	0	+

^a Symbols: +, positive; 0, negative.^b 1, Acetamide; 2, benzamide; 3, urea; 5, nicotinamide; 6, pyrazinamide; 9, succinamide.

narily is seen only among the nonpathogenic strains of group III (nonphotochromogenic) mycobacteria.

The susceptibility of the Malmö strains to ethambutol, ethionamide, kanamycin, and cycloserine in combination with resistance to the remaining drugs studied is remarkable. This pattern is not introduced by treatment, and it has not been previously reported.

Furthermore, the Malmö strains are distinguished by a unique surface antigen, a new lipid composition pattern, and a new type of sensitin. For these reasons, we regard these strains as belonging to a new species, for which we propose the name *Mycobacterium malmoense* (M.L. adj. *malmoensis* belonging to Malmö, Sweden, the source of the strains on which the original description of this species is based). Strain M-ö 816 is the type strain of *M. malmoense*; a culture of this strain has been deposited in the American Type Culture Collection under the number 29571.

Up to the present, *M. malmoense* strains have been isolated only from human beings. The isolates were pure cultures, and all of the patients had clinical and roentgenological signs of lung mycobacteriosis. No other mycobacteria or other pathogens were isolated from these patients. A report of the clinical data will be published separately in the near future.

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REPRINT REQUESTS

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