Isoniazid Susceptibility as a Criterion for the Differentiation of Mycobacterial Species from Other Mycolic Acid-Containing Taxa

P. A. B. ORLEAN, M. GOODFELLOW, and D. E. MINNIKIN

Departments of Microbiology and Organic Chemistry, The University, Newcastle upon Tyne, United Kingdom NE1 7RU

One hundred twenty-five strains of mainly fast-growing mycobacteria and representatives of related taxa were screened for susceptibility to 50, 100, and 200 μg of isoniazid per ml. Over 80% of the mycobacterial strains were susceptible to 200 μg of isoniazid per ml, with the resistant ones restricted to the species Mycobacterium album, M. aurum, M. chelonei, M. farrirogenes, and M. fortuitum. With a single exception, representatives of the taxa Bacterionema, Corynebacterium, Micromonospora, Nocardia, Rhodococcus, Streptomyces, and Gordona aurantiaca were resistant to the concentrations of isoniazid tested. The susceptibility of most fast-growing mycobacteria to high concentrations of isoniazid seems to provide the basis of a useful test to distinguish the majority of these organisms from related actinomycete and coryneform bacteria.

The status and relationships of the mycolic acid-containing taxa—Mycobacterium, Nocardia, Rhodococcus, Bacterionema, and Corynebacterium—have been clarified by using modern taxonomic methods such as numerical and chemical taxonomy (3, 5, 7). The genus Rhodococcus (4) was proposed for strains previously classified in the "rhodochrous" complex and the genus Gordona excepting the species Gordona aurantiaca, which was considered in need of further study. Despite the improvements in classification, few simple and reliable tests are available for the differentiation of mycobacterial species from allied taxa, although chemical tests are of some value in this respect (6, 7).

Isonicotinic acid hydrazide (isoniazid) susceptibility seems to be a characteristic of strains of Mycobacterium (1, 10), and resistance to 10 μg of this drug per ml has been developed as a reproducible test in the classification of mycobacteria (17). A current hypothesis on the mode of action of isoniazid suggests that it initially and specifically inhibits mycolic acid synthesis in Mycobacterium tuberculosis H37Ra and H37Rv (15; K. Takayama, H. K. Schnoes, and A. J. Valicenti, Fed. Proc. 35:724, 1976).

The present study sought to assess the use of isoniazid susceptibility as a character in the classification and identification of mycolic acid-containing taxa.

MATERIALS AND METHODS

One hundred twenty-five strains representing the genus Mycobacterium and related taxa were examined (see Table 1). The organisms were maintained at room temperature on modified Sauton agar slopes (8) supplemented with 0.1% (vol/vol) Tween 80 (Honeywell-Atlas Ltd., Carshalton, England). Susceptibility tests were performed on Bennett agar containing (grams per liter): yeast extract (Oxoid), 1; Lab Lemco (Oxoid), 0.8; N-Z Amine A (Sheffield Chemical Co., Norwich, N.Y.), 2; glucose, 10; aneurine hydrochloride, 0.05; Lab-M agar (pH 7.3) (Analytical & Bacteriological Media Ltd., London), 15, supplemented with 0.1% (vol/vol) Tween 80. Isoniazid (BDH Ltd., Poole, England) was added in aqueous solution and sterilized at 115°C for 15 min to give final concentrations of 50, 100, and 200 μg/ml.

Control and isoniazid-containing media were dispensed into divided polystyrene Replidishes (Sterilin Ltd., Teddington, England) and inoculated from drug-free master plates by using a multipoint inoculator (13). Inoculated dishes were examined for growth after 3 and 4 days of incubation at 30°C.

RESULTS

The susceptibility of the test strains to 50, 100, and 200 μg of isoniazid per ml in Bennett agar are given in Table 1. Most of the mycobacteria were susceptible to the isoniazid concentrations tested, and only strains of Mycobacterium album, M. aurum, M. chelonei, M. farrirogenes, M. fortuitum, and M. runyonii were resistant to 200 μg of the drug per ml. Except for a strain of Rhodococcus rhodochrous, the representatives of the taxa Bacterionema, Corynebacterium, Micromonospora, Nocardia, Rhodococcus, Streptomyces, and Gordona aurantiaca were resistant to isoniazid. The results were reproducible on three separate occasions.
**DISCUSSION**

The finding that isoniazid susceptibility is a characteristic of most of the mycobacteria examined is of interest in the differentiation of these organisms from those of allied taxa and in the context of the mode of action of the drug.

Of the *Mycobacterium* species containing strains resistant to 200 μg of isoniazid per ml, *M. chelonei* and *M. fortuitum* are established taxa, *M. runyonii* is a synonym of *M. chelonei*, and *M. album* and *M. aurum* are species incertae sedis (12). The remaining taxon, *M. farcinogenes*, was proposed for bacteria isolated from zebu with farcy in Africa (2). The strain of *M. farcinogenes* (N724) resistant to isoniazid was typical on the basis of serological data (11).

Isoniazid resistance may thus be confined to a few taxa in the genus *Mycobacterium* but is characteristic of strains classified in related actinomycete and coryneform taxa. The susceptibility of *Rhodococcus rhodochrous* N84 to isoniazid is difficult to explain, at present, for this organism was recovered in the same numerical phenetic cluster (4) and deoxyribonucleic acid homology group (9) as other strains of *R. rhodochrous*.

Isoniazid inhibits the incorporation of [14C]glycerol (18) and [14C]acetate (14, 16) into *Mycobacterium tuberculosis* H37Ra and H37Rv, respectively, and may selectively inhibit the desaturation mechanisms in *M. tuberculosis* H37Ra (15; Takayama et al., Fed. Proc. 35:724, 1976). However, from the present results it would seem that, if isoniazid acts on mycolic acid synthesis, then its effects are more or less confined to mycobacterial species. The susceptibility of fast-growing mycobacteria to high concentrations of isoniazid seems to provide the basis of a useful test to distinguish the majority of these organisms from related actinomycete and coryneform bacteria.

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**REPRINT REQUESTS**

Address reprint requests to: Dr. M. Goodfellow, Department of Microbiology, The Medical School, The University, Newcastle upon Tyne, United Kingdom NE1 7RU.

**LITERATURE CITED**


