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Resistance to Rifampin and Lysozyme of Strains of Some Species of *Mycobacterium* and *Nocardia* as a Taxonomic Tool

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Examination of 682 strains representing four species of mycobacteria (*Mycobacterium phlei, M. smegmatis, M. fortuitum,* and *M. marinum*) and seven species of nocardiae (*Nocardia asteroides, N. caviae, N. brasiliensis, N. autotrophica, N. dassonvillei, N. madurae,* and *N. pelletieri*) indicated that, with the exception of *N. madurae* and *N. pelletieri*, resistance to rifampin (20 μg/ml) is a useful property for describing these species. Resistance to lysozyme (300 to 500 U/ml) is taxonomically useful for delineating all 11 species.

Numerous studies have been made of the in vitro effect of rifampin, a derivative of the antibiotic rifamycin, on strains of *Mycobacterium tuberculosis* and other mycobacterial species (1-3, 6-25). The main purpose of these studies was to obtain indications of the effectiveness of the drug in the treatment of mycobacterial diseases. On the whole, these reports were in agreement on the susceptibility to rifampin of strains of *M. tuberculosis, M. bovis, M. kansasii,* and *M. phlei*. Strains of *M. fortuitum* and *M. smegmatis* were generally reported as resistant, whereas strains of *M. avium* (including *M. intracellulare*), *M. scrofulaceum,* and *M. marinum* varied in their susceptibility to the drug.

After their observations of the in vitro resistance to rifampin of 222 strains of mycobacteria, Rynearson et al. (20) suggested that the marked susceptibility of their 12 strains of *M. phlei* to rifampin indicated a useful means of identifying strains of this species.

As pointed out by Goodfellow and Orchard (4) in their review of reports on the susceptibility of actinomycetes to antibiotics, most of the studies of strains of nocardiae have emphasized the chemotherapeutic effect of the drugs rather than their value in taxonomy. In their own study of the taxonomic usefulness of antibiotic susceptibility, Goodfellow and Orchard (4) reported that 30 strains of *Nocardia asteroides* and 28 of *N. brasiliensis* were resistant to rifampin in a concentration of 50 μg/ml. Of 15 strains of *N. caviae,* 8 were resistant to the same concentration of the drug.

Following these suggestions (4, 20), we tested a number of our strains of mycobacteria and nocardiae for their resistance to rifampin and now routinely apply the test to all the strains we examine.

Resistance to lysozyme is another test routinely applied in our taxonomic studies. We previously reported (5) our findings on the resistance to lysozyme of strains of nocardiae but, to date, we have not described the resistance of our strains of mycobacteria. Therefore, we are presenting here the results of our observations on the resistance of strains of some species of *Mycobacterium* and *Nocardia* to rifampin and to lysozyme.

MATERIALS AND METHODS

Organisms. The species of *Mycobacterium* and *Nocardia* and the number of strains representing each species are given in Table 1. A list of the 682 strains examined, their strain designations, and their histories is available from the authors.

Resistance to rifampin. A stock solution of rifampin (1 mg/ml) was prepared by dissolving 10.2 mg of rifampin (potency 981 μg/mg, from Ciba-Geigy Corp., Summit, N. J.) in 2 ml of dimethyl sulfoxide and bringing the volume up to 10 ml in a volumetric flask with sterile distilled water. The resulting stock solution was sterilized by filtration through a 0.2-μm membrane filter. The stock solution was stored at 3 to 4°C, used for 1 month, and then replaced, when necessary, with freshly prepared solution.

Susceptibility of the cultures to 20 μg of rifampin per ml was determined by diluting 2 ml of the stock solution to 100 ml in a volumetric flask with sterile glucose broth (5 g each of peptone, beef extract, glucose, and yeast extract per liter of distilled water; pH 7.0). This rifampin solution in glucose broth was pipetted aseptically in 2.5-ml amounts into sterile capped tubes. Cultures in glucose broth, approximately 3 weeks old, were inoculated with a small loop into tubes of rifampin broth and of glucose broth (control) in such a way that the inoculum could not be mistaken later for growth. The cultures were incubated at 28°C and examined for growth at 1 and 2 weeks.

Resistance to lysozyme. A solution of lysozyme

TABLE 1. *Resistance of strains of mycobacteria and nocardiae to rifampin and lysozyme*

Species	No. of strains[a]	Resistant to:	
		Rifampin (% positive)	Lysozyme (% positive)
Mycobacterium phlei	41	5	0
M. smegmatis	81	97	9
M. fortuitum	115	100	99
M. marinum	62	0	100
Nocardia asteroides	141	96	100
N. caviae	35	100	100
N. brasiliensis	54	100	100
N. autotrophica	31	0	0
N. dassonvillei	49	0	0
N. madurae	49	65	8
N. pelletieri	24	46	0

[a] Strain designations and histories of the 682 strains examined are available from the authors upon request.

was made by dissolving 0.1 g of lysozyme (6,000 to 10,000 U/mg, from Nutritional Biochemicals Corp., Cleveland, Ohio) in 100 ml of sterile distilled water in a volumetric flask and sterilizing the solution by filtration. This stock solution was stored at 3 to 4°C and discarded after 2 weeks. A 5-ml amount of the stock lysozyme solution was mixed with 95 ml of sterile glycerol broth (peptone, 5 g; beef extract, 3 g; glycerol, 70 ml; distilled water, 1,000 ml; pH 7.0) and dispensed aseptically in sterile capped tubes. Tubes of lysozyme broth (300 to 500 U/ml) and glycerol broth (control) were inoculated as in the above test for resistance to rifampin. After 2 and 4 weeks of incubation at 28°C, the cultures were observed for growth.

RESULTS

Our data on the resistance to rifampin (20 μg/ml) and to lysozyme (300 to 500 U/ml) of 682 strains representing four species of mycobacteria and seven species of nocardiae are presented in Table 1. Among 41 strains of *M. phlei*, only two strains were found to be resistant to rifampin, and no strains were found to be resistant to lysozyme. All but 2 of 81 strains of *M. smegmatis* were resistant to rifampin, whereas 7 were resistant to lysozyme. All 115 strains of *M. fortuitum* grew in 20 μg of rifampin per ml and, with one exception, all grew in 300 to 500 U of lysozyme per ml. Rifampin inhibited growth of 62 strains of *M. marinum*, but all 62 strains grew in lysozyme.

Among the nocardiae examined, 96% of 141 strains of *N. asteroides* were resistant to rifampin and all 141 strains were resistant to lysozyme. All 35 strains of *N. caviae* and 54 of *N. brasiliensis* were resistant to rifampin and lysozyme. Both rifampin and lysozyme prevented growth of 31 strains of *N. autotrophica* and 49 strains of *N. dassonvillei*. Strains of *N. madurae* and *N. pelletieri* (49 and 24 strains, respectively) varied in their resistance to rifampin but were generally sensitive to lysozyme.

DISCUSSION

A taxonomist's search for criteria to improve his descriptions of species and his means of identifying strains is never-ending. In this continuous search, the taxonomic value of a test can be established only by its application to many strains. In our experience also, a test that is very useful in delineating some species is not necessarily useful in characterizing other species of the same genus.

Insofar as the representation in our collection of the various species indicates, resistance or sensitivity to rifampin (20 μg/ml) can be used in the description and recognition of strains of the four species of mycobacteria and of five of the seven species of nocardiae examined here; resistance or susceptibility to rifampin is acceptable as one of a group of properties used to define these nine species. Under the conditions of our test, resistance to rifampin by strains of *N. madurae* and *N. pelletieri* is a variable characteristic and taxonomically useless. On the other hand, resistance or susceptibility to lysozyme (300 to 500 U/ml) appears to be a taxonomically valuable property for all the species listed here.

Our results are presented here only for their taxonomic worth; they are not intended to have any therapeutic significance. We apply 50 other tests and observations to our unknown strains. The simple test for resistance or susceptibility to rifampin and to lysozyme, as described here, is feasible in our laboratory, whereas a more time-consuming procedure could not be undertaken.

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REPRINT REQUESTS

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