SHORT COMMUNICATION

Differentiation between the Genera *Mycobacterium*, *Rhodococcus* and *Nocardia* by Susceptibility to 5-Fluorouracil

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A test for susceptibility to 5-fluorouracil was useful for differentiating between species of rapidly growing mycobacteria, and for differentiating the genus *Rhodococcus* from the genus *Nocardia*. The majority of rhodococci tested were susceptible to 5-fluorouracil ($20\text{ }\mu\text{g ml}^{-1}$), whereas the majority of nocardiae tested were resistant to it. Strains of *Nocardia asteroides sensu stricto* could be divided into two subgroups by their reaction to 5-fluorouracil.

INTRODUCTION

Tsukamura (1971) proposed a new genus, '*Gordona*', for slightly acid-fast organisms occurring in sputa of patients with pulmonary disease, and in soil. Subsequently, Tsukamura (1973) included '*Mycobacterium*' *rhodochrous* (Gordon & Mihm, 1957; Gordon, 1966) as a member of '*Gordona*', together with a new species, '*Gordona*' *aurantiaca* (Tsukamura & Mizuno, 1971). Tsukamura (1974*a*) proposed the revival of *Rhodococcus* as the generic name for this taxon, and the following six species were described: *R. rhodochrous*, *R. bronchialis*, *R. rubropertinctus*, *R. terrae*, '*R. roseus*' and '*R. aurantiacus*'. Goodfellow & Alderson (1977) carried out an extensive study on these organisms and formally defined the genus *Rhodococcus*. *Rhodococcus* is closely related to *Nocardia* and, to a lesser extent, to *Mycobacterium* (Tsukamura, 1971); a number of characteristics which are useful for differentiating these three genera have been studied (Tsukamura, 1971, 1974*a, b*; Goodfellow & Alderson, 1977; Tsukamura *et al.*, 1979). However, it is still important to establish some simple definitive tests for differentiating *Rhodococcus* from *Nocardia*. In the present study, susceptibility to 5-fluorouracil was shown to achieve this, and also to differentiate between species of rapidly growing mycobacteria and subgroups of *Nocardia asteroides*.

METHODS

The strains used were ones maintained in this laboratory. They were cultivated on Ogawa egg medium at $37\text{ }^{\circ}\text{C}$ for 3 d (*M. flavescens* and *M. thermoresistibile* for 5 d) for use as inocula. The composition of the Ogawa egg medium was: basal solution [1% (w/v) KH_2PO_4 and 1% (w/v) sodium glutamate], 100 ml; whole eggs, 200 ml; glycerol, 6 ml; 2% (w/v) aqueous solution of malachite green, 6 ml (resulting pH 6.8). 5-Fluorouracil (Kyowa Fermentation Co., Tokyo) was dissolved in propylene glycol to give a concentration of 10 mg ml^{-1} . This solution was diluted with distilled water to give concentrations of 4.0, 2.0, 1.0 and 0.5 mg ml^{-1} , and 1 vol. of each dilution was added to 100 vol. Ogawa egg medium before sterilization, giving final concentrations of 5-fluorouracil of 40, 20, 10 and 5 $\mu\text{g ml}^{-1}$. The medium was poured in 7 ml portions into tubes ($165 \times 16.5\text{ mm}$), which were sloped after sterilization at $90\text{ }^{\circ}\text{C}$ for 60 min.

One loopful (about 0.1 mg wet wt) of the test strain was inoculated on to the Ogawa egg medium containing the various concentrations of 5-fluorouracil. After inoculation, each tube was loosely stoppered with a double-edged gum cap having a 3 mm cut in its centre, and incubated at $37\text{ }^{\circ}\text{C}$ for 3 d (*M. flavescens* and *M. thermoresistibile* for 5 d). Positive growth was regarded as growth similar to that on control medium, which contained no 5-fluorouracil.

RESULTS AND DISCUSSION

The results obtained are shown in Table 1. The nocardiae were resistant to 5-fluorouracil at 40 $\mu\text{g ml}^{-1}$ (except for some strains of *N. asteroides* and *N. brasiliensis*); most of the rhodococci, however, were susceptible to 5 $\mu\text{g ml}^{-1}$, although some strains of *R. rubropertinctus*, *R. corallinus* and *R. terrae* were somewhat less susceptible. Most of the rhodococci could be differentiated from most of the nocardiae by their susceptibility to

Table 1. Tolerance to 5-fluorouracil of rapidly growing mycobacteria, rhodococci and nocardiae

Species	No. of strains tested	No. of strains showing growth				
		5-Fluorouracil concn ($\mu\text{g ml}^{-1}$)				
		40	20	10	5	0
<i>Mycobacterium</i>						
Rapidly growing, non-photochromogenic						
<i>M. fortuitum</i>	10	10	10	10	10	10
<i>M. chelonae</i> subsp. <i>chelonae</i>	10	10	10	10	10	10
<i>M. chelonae</i> subsp. <i>abscessus</i>	10	10	10	10	10	10
<i>M. smegmatis</i>	5	5	5	5	5	5
<i>M. chitae</i>	5	0	0	0	0	5
' <i>M. agri</i> '	5	0	0	0	0	5
Rapidly growing, photochromogenic						
<i>M. vaccae</i>	5	0	0	1	1	5
<i>M. parafortuitum</i>	3	2	3	3	3	3
Rapidly growing, scotochromogenic						
<i>M. flavescens</i>	5	0	0	0	0	5
<i>M. thermoresistibile</i>	5	0	0	0	0	5
<i>M. phlei</i>	5	0	0	0	0	5
<i>M. aurum</i>	5	0	0	0	0	5
<i>M. neoaurum</i>	5	0	0	0	0	5
' <i>M. obuense</i> '	5	0	0	0	0	5
' <i>M. aichiense</i> '	5	0	0	0	0	5
' <i>M. chubuense</i> '	5	0	0	0	0	5
' <i>M. rhodesiae</i> '	5	0	0	0	0	5
Kanazawa strains (see text)	2	0	0	0	0	2
<i>Rhodococcus</i>						
<i>R. rhodochrous</i>	8	0	0	0	0	8
<i>R. bronchialis</i>	10	0	0	0	0	10
<i>R. rubropertinctus</i>	10	0	0	2	3	10
<i>R. terrae</i>	10	0	7*	8	8	10
<i>R. ruber</i> (' <i>R. lentifragmentus</i> ')†	5	0	0	0	0	5
<i>R. corallinus</i>	2	0	0	1	2	2
<i>R. equi</i>	2	0	0	0	0	2
<i>R. coprophilus</i>	2	0	0	0	0	2
<i>Nocardia</i>						
<i>N. asteroides</i> subgroup A	10	9	10	10	10	10
<i>N. asteroides</i> subgroup B	11	0	0	2	5	11
<i>N. farcinica</i>	10	10	10	10	10	10
<i>N. brasiliensis</i> ‡	5	3	3	3	3	5
<i>N. otitidis-caviarum</i>	8	8	8	8	8	8
<i>N. autotrophica</i>	2	2	2	2	2	2
' <i>Gordona</i> ' <i>aurantiaca</i>	10	10	10	10	10	10

* Slight growth.

† See Tsukamura *et al.* (1975).

‡ Strains resistant to 40 $\mu\text{g ml}^{-1}$ were 23083 (M199), 23104 (R405) and 23106 (R887); strains sensitive to 5 $\mu\text{g ml}^{-1}$ were 23086 (M204) and 23105 (R432).

Table 2. Strains of *Nocardia asteroides* placed into subgroups A and B on the basis of susceptibility to 5-fluorouracil

	Strain no.	Source*
Subgroup A	23007†	I. Uesaka; N. M. McClung, M94‡
	23012†	I. Uesaka; N. M. McClung, M145
	23039†	I. Uesaka; N. M. McClung, M94‡
	23046†	I. Uesaka; N. M. McClung, M129
	23049†	I. Uesaka; N. M. McClung, M132
	23064	I. Uesaka; N. M. McClung, M161
	23094	R. E. Gordon, R399 (ATCC 23824)§
	23099	R. E. Gordon, R860
	23206	I. J. Bousfield, P29; ATCC 19247, type strain
	23202	M. Tsukamura, 9742
Subgroup B	23006†	I. Uesaka; N. M. McClung, M93‡
	23019	I. Uesaka; N. M. McClung, M78
	23032†	I. Uesaka; N. M. McClung, M10
	23038†	I. Uesaka; N. M. McClung, M93‡
	23044†	I. Uesaka; N. M. McClung, M124
	23088†	I. Uesaka; N. M. McClung, M206
	23095†	R. E. Gordon, R443(1)
	23096	R. E. Gordon, R443(2)
	23203	M. Tsukamura, 9867
	23204	M. Tsukamura, 9868
	23205	M. Tsukamura, 9869

* I. Uesaka, Kyoto University, Kyoto, Japan; N. M. McClung, University of South Florida, Tampa, Florida, U.S.A.; R. E. Gordon, Rutgers University, New Brunswick, New Jersey, U.S.A.; I. J. Bousfield, Torry Research Station, Aberdeen, Scotland.

† Strains placed into subgroups A and B by Tsukamura (1977) and Tsukamura *et al.* (1979).

‡ Strains received from Dr I. Uesaka.

§ Strain deposited in the American Type Culture Collection by Tsukamura (1969) as reference strain.

5-fluorouracil at 20 $\mu\text{g ml}^{-1}$. The strains of *Nocardia asteroides* could be divided into those tolerant to 5-fluorouracil at 20 $\mu\text{g ml}^{-1}$ (subgroup A) and those susceptible at this concentration (subgroup B). The rapidly growing mycobacteria could also be divided into two distinct groups: those tolerant to 20–40 $\mu\text{g ml}^{-1}$ and those susceptible to 5 $\mu\text{g ml}^{-1}$.

Susceptibility to 5-fluorouracil is therefore considered to be a useful tool for differentiating *Rhodococcus* from *Nocardia*, the former being more susceptible than the latter. '*Gordona aurantiaca*' (Tsukamura & Mizuno, 1971) was considered not to belong to *Rhodococcus* or *Nocardia* (Goodfellow *et al.*, 1978; Tsukamura *et al.*, 1979). This taxon could be distinguished from rhodococci but not from nocardiae by this test.

Previously, strains of *N. asteroides* were considered as homogeneous (Gordon & Mihm, 1957, 1962). Tsukamura (1969) divided strains received as *N. asteroides* into two distinct taxa, *N. asteroides sensu stricto* and *N. farcinica*, the latter containing strain ATCC 3318 (the type strain of *N. farcinica*). In a subsequent study (Tsukamura, 1977), the taxon *N. asteroides sensu stricto* itself appeared to be divided into two subgroups, A and B. In a numerical analysis of nocardiae, rhodococci and mycobacteria, subgroup A appeared distinct from rhodococci and closely related to the other nocardiae, while subgroup B was intermediate between *Rhodococcus* and *Nocardia* (Tsukamura *et al.*, 1979). The subgroups A and B of *N. asteroides* defined in these previous studies corresponded to those distinguished here (Table 2). Subgroup A, previously shown to be closely related to other nocardiae, was tolerant to 5-fluorouracil as were other nocardiae. In contrast, subgroup B, previously shown to be related to rhodococci, was more susceptible to 5-fluorouracil as were the rhodococci tested.

Previously, Tsukamura (1979) tested the susceptibility to 5-fluorouracil of slowly growing mycobacteria and observed that almost all slowly growing mycobacteria, except *M. triviale*, were relatively susceptible to this agent. In the present work, rapidly growing mycobacteria were studied. One group was tolerant to 20–40 µg ml⁻¹ and contained *M. fortuitum*, *M. chelonae* subsp. *chelonae*, *M. chelonae* subsp. *abscessus*, *M. smegmatis* and *M. parafortuitum*. The other group was susceptible to 5 µg ml⁻¹ and contained *M. flavescens*, *M. thermoresistibile*, *M. phlei*, *M. chitae*, '*M. agri*', *M. aurum*, *M. neoaurum*, '*M. obuense*', *M. vaccae*, '*M. aichiense*', '*M. chubuense*' and '*M. rhodesiae*'. The 'Kanazawa strains' had previously been identified as *M. parafortuitum* by Tsukamura (unpublished). However, these were suggested to be distinct from *M. parafortuitum* by Saito *et al.* (1977). The results here support this conclusion.

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