Differentiation of the '*Mycobacterium*' *rhodochrous*-group from *Nocardiae* by β -Galactosidase Activity

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INTRODUCTION

Gordon & Mihm (1957; 1959) differentiated '*Mycobacterium*' *rhodochrous* from the genus *Nocardia* and considered the taxon to be a member of the genus *Mycobacterium*. Later, Gordon (1966) reconsidered this classification, suggesting the organism occupied an intermediate position between *Mycobacterium* and *Nocardia*. The organisms are currently referred to as '*Mycobacterium*' *rhodochrous*, the correct genus remaining undecided.

Tsukamura (1971) isolated a number of slightly acid-fast organisms, similar to '*Mycobacterium*' *rhodochrous* but not identical, from sputa of patients with pulmonary disease and from soil, and proposed a new genus, *Gordona*, for them. Six species of the genus *Gordona* have been described: *G. bronchialis* (Tsukamura, Inagaki & Kondo, 1970; Tsukamura, 1971); *G. rubropertincta* (synonym, *G. rubra*) (Tsukamura, 1971; 1973); *G. terrae* (Tsukamura, 1971); *G. aurantiaca* (Tsukamura & Mizuno, 1971); *G. rhodochroa* (Tsukamura, 1973); and *G. rosea* (Tsukamura, 1973). The last two species had previously been named '*M.*' *rhodochrous* but have now been included in the new genus (Tsukamura, 1971); Tsukamura (1971) described eight characters for distinguishing the genus *Gordona* from the genera *Mycobacterium* and *Nocardia*. The present study reports that the test for β -galactosidase activity is also useful for distinguishing the genus *Gordona* from the genus *Nocardia*. Because the genus *Gordona* is not yet generally accepted, it is called the '*rhodochrous*-group' in the present study.

METHODS

A modification of the methods of Tacquet *et al.* (1966) and of Kubala (E. Kubala, personal communication) was used for testing β -galactosidase activity.

The basal medium contained: KH_2PO_4 , 1.0 g; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 6.25 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.6 g; sodium citrate, 1.5 g; asparagine, 2.0 g; 10% (v/v) Tween 80 solution, 5.0 ml; and distilled water, 1000 ml, and was sterilized by autoclaving. To 100 ml of this medium, 0.1 g of 2-nitrophenyl- β -D-galactopyranoside (BDH Chemicals, Poole, Dorset) was added, and the medium was sterilized by heating at 100 °C for 5 min in a water bath (the heating time should be exactly 5 min because excess heating produces a yellow colouring in the medium); the medium may also be sterilized by filtration. After cooling, bovine serum (Eiken Co., Japan, Tokyo) was added to give a final concentration of 4% (v/v).

Tubes containing 5 ml of the complete medium were inoculated with one loopful (about 1 mg) of test organism and incubated at 28 °C for 7 days. A positive reaction was indicated by a yellow coloration not evident in the uninoculated control tube. The readings were clear-cut and reproducible. In three replications made at different times, all the *rhodochrous*-group test strains, except two strains of *Gordona bronchialis*, showed the same reaction.

Table 1. *β -Galactosidase activity of nocardiae, rhodochrous-group (Gordona) organisms and rapidly growing mycobacteria*

	No. of strains tested	No. of strains showing a positive reaction	No. of strains showing a negative reaction
<i>N. asteroides</i>	14	14	0
<i>N. brasiliensis</i>	9	9	0
<i>N. caviae</i>	8	8	0
<i>N. farcinica</i>	34	34	0
<i>N. rubra</i> *	6	2	4
<i>N. pelletieri</i>	5	0	5
<i>N. madurae</i> †	4	1	3
<i>G. rhodochroa</i> ‡	10	0	10
<i>G. bronchialis</i>	33	2	31
<i>G. rubropertincta</i> (<i>G. rubra</i>)	14	0	14
<i>G. terrae</i>	14	0	14
<i>G. rosea</i> §	2	0	2
<i>G. aurantiaca</i>	4	4	0
<i>M. smegmatis</i>	20	20	0
<i>M. phlei</i>	21	16	5
<i>M. chelonae</i> subsp. <i>chelonae</i>	20	20	0
<i>M. chelonae</i> subsp. <i>abscessus</i>	20	19	1
<i>M. fortuitum</i>	20	0	20
<i>M. chitae</i>	6	0	6
<i>M. vaccae</i>	13	0	13
<i>M. aurum</i>	36	3	33
<i>M. parafortuitum</i>	13	0	13
<i>M. rhodesiae</i>	21	3	18
<i>M. neoaurum</i>	20	1	19
<i>M. aichiense</i>	8	0	8
<i>M. chubuense</i>	18	2	16
<i>M. flavescens</i>	10	0	10
<i>M. thermoresistibile</i>	10	0	10

* Strains M6 and M75 showed a positive reaction, and strains M1, M103, M191 and M192 showed a negative reaction.

† Strain R431 showed a positive reaction, and strains R706, R1091 and R1260 showed a negative reaction.

‡ Ten strains of *G. rhodochroa* were received as '*M.*' *rhodochrous*: ATCC 14348; ATCC 4001; ATCC 999; ATCC 14349; ATCC 14341; ATCC 4276; ATCC 4273; ATCC 14347; N31 (*M. Goodfellow*); ATCC 13808.

§ Two strains of *G. rosea* were received as '*M.*' *rhodochrous*: ATCC 271; ATCC 4004.

RESULTS AND DISCUSSION

Five of the six species of the genus *Gordona* (the *rhodochrous*-group), including all strains received as '*M.*' *rhodochrous*, showed a negative reaction in the test (Table 1). Only *G. aurantiaca* showed a positive reaction. Unlike the others, this species was acid-fast with orange pigmented colonies, and had been regarded as an outsider of the group (Tsukamura & Mizuno, 1971).

All 'true' nocardiae, *Nocardia asteroides*, *N. brasiliensis* and *N. caviae*, showed a positive reaction. Therefore the *rhodochrous*-group could be differentiated from the *Nocardia* by its absence of β -galactosidase activity.

Nocardia pelletieri and *N. madurae* showed a negative reaction. These two species had been distinguished from the 'true' nocardiae as 'streptomyces-like' nocardiae (Schneidau

& Shaffer, 1957; 1960) and have been included in a different genus, *Actinomadura* (Lechevalier & Lechevalier, 1970). *Nocardia farcinica* showed a positive reaction. A group of strains received as nocardiae and provisionally classified as the species *Nocardia rubra* (Tsukamura, 1969) showed different reactions.

Rapidly growing mycobacteria were separated into two subgroups: *Mycobacterium smegmatis*, *M. phlei* and *M. chelonae* showed a positive reaction; *M. fortuitum*, *M. chitae*, *M. thermoresistibile*, *M. flavescens*, *M. vaccae*, *M. aurum*, *M. parafortuitum*, and the recently defined, rapidly growing, scotochromogenic mycobacteria, *M. rhodesiae*, *M. neoaurum*, *M. aichiense* and *M. chubuense*, gave a negative reaction.

The test for β -galactosidase activity is useful for differentiating the *rhodochrous*-group (the genus *Gordona*) from the genus *Nocardia*.

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