Differentiation of *Rhodococcus* (*Gordona*) and *Nocardia* by Thin-layer Chromatography after Uptake of [³⁵S]Methionine

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INTRODUCTION

Tsukamura (1971) proposed the genus *Gordona* for slightly acid-fast organisms isolated from sputa of patients and soil. Since the genus was found to contain the type strain of *Rhodococcus rhodochrous* (ATCC 13808), the gordonae were reclassified in the genus *Rhodococcus* (Tsukamura, 1974), a proposal supported by Goodfellow & Alderson (1977). Previously, Tsukamura & Mizuno (1975) reported that the thin-layer chromatography of ethanol/diethyl ether extracts, after uptake of [³⁵S]methionine, was useful for the differentiation of mycobacterial species. We have now found that this technique is also useful for the differentiation of *Rhodococcus* and *Nocardia*.

METHODS

The following strains were used: *Nocardia asteroides*: 23007 (M-94); 23009 (M-124); 23032 (M-10); *N. farcinica* (see Tsukamura, 1969): 23036 (M-81); 23047 (M-130); 23102 (ATCC 3318); *N. brasiliensis*: 23086 (M-204); 23106 (R-887); 23109 (R-1188); *N. caviae*: 23035 (M-73); 23078 (M-185); 23113 (R-617); *Rhodococcus lentifragmentus* [according to Goodfellow & Alderson (1977), this organism should be called *Rhodococcus ruber*; synonyms are *N. rubra* and *Gordona lentifragmenta* (Tsukamura, Mizuno & Murata, 1975)]: 23002 (M-1); 23022 (M-122); 23024 (M-192); *R. rhodochrous* (Tsukamura, 1973, 1974; Goodfellow & Alderson, 1977): 40001 (ATCC 13808); 40002 (M. Goodfellow, M-30); 40003 (M. Goodfellow, tsu); 40012 (ATCC 25970); 40013 (ATCC 25971); 40015 (ATCC 4001); 40016 (ATCC 4276); 40017 (ATCC 4273); 40019 (ATCC 14341); 40020 (ATCC 14347); 40021 (ATCC 14348); 40022 (ATCC 14349); *R. bronchialis* (syn. *G. bronchialis*) (Tsukamura 1971, 1974): 50003 (ATCC 25592, NCTC 10667); 50006; 50011; 50013; 50017; 50019; 50051; 50093; 50094; 50095; 50096; *R. rubropertinctus* (syn. *G. rubropertincta*) (Tsukamura, 1971, 1973, 1974): 6001; 60002; 60003 (ATCC 25593, NCTC 10668); 60004; 60005; 60015; 60016; 60017; 60018; 60020; *R. terrae* (syn. *G. terrae*) (Tsukamura, 1971, 1974): 70001; 70002; 70003; 70004; 70005; 70006 (ATCC 25594, NCTC 10669); 70007; 70008; 70009; 70010; *R. aurantiacus* (syn. *G. aurantiaca*) (Tsukamura & Mizuno, 1971; Tsukamura, 1974): 80001 (ATCC 25938; NCTC 10741); 80003; 80004. Strains with the prefix M were received from Dr N. M. McClung through Dr I. Uesaka, Kyoto University, and those with the prefix R and strain ATCC 3318 were from R. E. Gordon, Rutgers University, U.S.A. The ATCC strains were from the American Type Culture Collection. The *Rhodococcus* strains examined were those previously called *Gordona*; Goodfellow & Alderson (1977) described several other species.

The experimental methods used were similar to those described previously for rapidly growing mycobacteria (Tsukamura & Mizuno, 1975), and, as before, Ogawa egg medium was used for harvesting the test organisms. The previous methods were modified in that L-[³⁵S]methionine was used at 10 μ Ci ml⁻¹ and the nocardiae were harvested at 28 °C.

RESULTS AND DISCUSSION

Thin-layer chromatograms of ethanol/diethyl ether extracts of nocardiae showed only one radioactive spot at R_F value 0.15 to 0.18 (Fig. 1), but those of the rhodococci had two radioactive spots at R_F values 0.15 to 0.18 and 0.30 to 0.35 (Fig. 1). Test organisms belonging to the same genus showed similar patterns, with the exception of four strains: *R. rhodochrous* 40003 and *R. terrae* 70010 showed only a single spot at R_F value 0.16, and *R. rhodochrous* 40015 and *R. terrae* 70009 showed a single spot at R_F value 0.31.

Fig. 1. Distribution of radioactive spots in thin-layer chromatograms of ethanol/diethyl ether extracts of nocardiae and rhodococci: (a) *N. asteroides* 23007; (b) *N. brasiliensis* 23086; (c) *N. caviae* 23035; (d) *R. bronchialis* 50003; (e) *R. terrae* 70004; (f) *R. aurantiacus* 80003; (g) *R. rhodochrous* 40022.

The cells were extracted twice with 2.0 ml 10% (w/v) trichloroacetic acid and then twice with 3.0 ml ethanol/diethyl ether (1:1, v/v). The ethanol/diethyl ether extracts were concentrated to 0.1 ml under reduced pressure, and the concentrates were chromatographed on thin-layers (0.25 mm thick) of Silica Gel H using 1-propanol/1-butanol/water/ammonia (57:20:20:3, by vol.) as solvent. (The origin of the chromatographs is on the right.)

Thus, representatives of the genera *Rhodococcus* and *Nocardia* showed almost genus-specific patterns in contrast to the almost species-specific patterns found with mycobacteria (Tsukamura & Mizuno, 1975). A small difference was observed between *R. rhodochrous* and other rhodococci previously called *Gordona*; *R. rhodochrous* lacked a distinct spot at R_F value 1.00 (front) which was present in the other rhodococci. *Rhodococcus lentifragmentus* previously named *Nocardia rubra* (Tsukamura, 1969) was transferred to the genus *Gordona* (now, *Rhodococcus*) by Tsukamura *et al.* (1975); this organism showed the same pattern as other species of *Rhodococcus*.

Thus, thin-layer chromatography using [35 S]methionine seems to be useful for differentiating between the genera *Rhodococcus* and *Nocardia*.

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