Rhodococcus sputi sp. nov., nom. rev., and *Rhodococcus aurantiacus* sp. nov., nom. rev.

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***Rhodococcus sputi* (Tsukamura 1978) and *Rhodococcus aurantiacus* (Tsukamura and Mizuno 1971; Tsukamura 1974) were validly and effectively published but were not included on the Approved Lists of Bacterial Names. The names are formally revived.**

In 1971 Tsukamura (16) proposed the generic name “*Gordona*” for slightly acid-fast organisms that were isolated from sputa of patients and from soil. At that time, the following three species were described: “*Gordona bronchialis*,” “*Gordona rubra*,” and “*Gordona terrae*.” Later, after it was shown that this genus also contained the species *Rhodococcus rhodochrous*, it was proposed that members of the genus “*Gordona*” be transferred to the genus *Rhodococcus* (17). Tsukamura (17) described the following six species: *Rhodococcus bronchialis*, *Rhodococcus rubropertinctus*, *Rhodococcus terrae*, *R. rhodochrous*, *Rhodococcus aurantiacus*, and “*Rhodococcus roseus*” (17). Goodfellow and Alderson (3, 6, 13) described three additional species (*Rhodococcus erythropolis*, *Rhodococcus rhodonii*, and *Rhodococcus equi*), and Rowbotham and Cross (11) described *Rhodococcus coprophilus*. In 1978 a full description of the genus *Rhodococcus* was published, and at the same time another species, *Rhodococcus sputi*, was described (20). More recently, Nesterenko et al. (10) described *Rhodococcus maris* and *Rhodococcus luteus*, and Tsukamura (7, 25) added three species (*Rhodococcus aichiensis*, *Rhodococcus chubuensis*, and *Rhodococcus obuensis*).

The morphological and physiological characteristics of the genus *Rhodococcus* have been described by Tsukamura (16, 20). Members of this genus contain mycolic acids with 30 to 66 carbon atoms and on this basis can be differentiated from members of the genus *Mycobacterium*, which have mycolic acids with 60 to 90 carbon atoms (1, 2, 4, 8, 9, 12, 14, 30, 31). Rhodococci are also differentiated from mycobacteria by the absence of strong acid fastness and the absence of arylsulfatase activity (16). However, rhodococci are not convincingly differentiated from nocardiae by mycolic acid analysis, because members of these two genera produce the same mycolic acid pattern in thin-layer chromatograms (4, 8, 14, 30, 31) and because the mycolic acids of rhodococci and nocardiae have the same range of carbon atom numbers (14, 21, 30, 31). Rhodococci may be differentiated from nocardiae by other characteristics, including the following three tests recently described by Tsukamura (22–24): susceptibility to 5-fluorouracil, susceptibility to mitomycin C, and susceptibility to bleomycin.

The bacteria named *R. sputi* and *R. aurantiacus* (17, 20, 28) (names which were not included on the Approved Lists of Bacterial Names [13]) possess the characteristics of the genus *Rhodococcus* but are sufficiently distinct to warrant separate species status (25, 29). The name *Rhodococcus*

sputi was published validly in 1978 (20) but unfortunately was omitted from the Approved Lists (13). This name should be revived.

The species *R. aurantiacus* was first described by Tsukamura and Mizuno (28) in 1971 as “*Gordona aurantiaca*.” When Tsukamura (17) proposed to change the generic name “*Gordona*” to *Rhodococcus*, the species was named *Rhodococcus aurantiacus*. In 1978, when Tsukamura (20) published the generic name *Rhodococcus* validly in the International Journal of Systematic Bacteriology, the name *Rhodococcus aurantiacus* was also published. Nevertheless, this name was also omitted from the Approved Lists (13). This name should be revived. However, in this case further explanation and justification are required. When Tsukamura and Mizuno (28) included this taxon in the genus “*Gordona*,” the taxon was considered to be somewhat irregular, because the bacteria showed stronger acid fastness than other members of the genus “*Gordona*” and also did not reduce nitrate. In 1974, Tsukamura (18) observed that members of the species were exceptionally β -galactosidase positive, although other rhodococci were β -galactosidase negative. In the same year, Tsukamura (17) included this taxon in the genus *Rhodococcus*, but noted that it was somewhat distinct from the other species in the genus (*R. rhodochrous*, *R. bronchialis*, *R. rubropertinctus*, *R. terrae*, and “*R. roseus*”). Goodfellow et al. (5) and Tomiyasu and Yano (14) observed that *R. aurantiacus* contained some characteristic mycolic acids and completely unsaturated menaquinones, and, on the basis of these findings, Goodfellow et al. (5) suggested that the species might belong to another genus. In the numerical taxonomic study of Tsukamura et al. (29), *R. aurantiacus* strains clustered outside the group composed of *R. rhodochrous*, *R. bronchialis*, *R. rubropertinctus*, *R. terrae*, “*R. roseus*,” and *Rhodococcus lentifragmentus* (the name *R. lentifragmentus* is used because it has priority over *Rhodococcus ruber* [13, 26]). Later, Tsukamura (22–24) noted that rhodococci were susceptible to 5-fluorouracil, mitomycin C, and bleomycin, whereas nocardiae were resistant. *R. aurantiacus* was also susceptible to bleomycin, but was resistant to the other two compounds. In 1982, Tsukamura (25) performed a more extensive numerical taxonomic study, in which *R. aurantiacus* again clustered outside the large group formed by the “classic” rhodococci of Tsukamura, (*R. rhodochrous*, *R. bronchialis*, *R. terrae*, *R. rubropertinctus* [17], *R. sputi*, and *R. lentifragmentus* [20]) and the three newly described species (*R. aichiensis*, *R. chubuensis*, and *R. obuensis*) (7, 25). However, the species described by Goodfellow and Alderson (3) (*R. equi*, *R. erythropolis*, and

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R. rhodnii) and *R. coprophilus* Rowbotham and Cross (11) were more distantly related to the large rhodochrous group than strains of *R. aurantiacus* were.

R. aurantiacus should be included in the genus *Rhodococcus* for the following reasons: (i) except for β -galactosidase activity and resistance to 5-fluorouracil and mitomycin C, *R. aurantiacus* strains conform to the description of the genus *Rhodococcus* given by Tsukamura (20); (ii) in the numerical taxonomic study of Tsukamura (25), strains of this species clustered as a distinct group among the other species of the genus *Rhodococcus*; and (iii) the name *R. aurantiacus* was published validly in 1974 (17) and appeared in the International Journal of Systematic Bacteriology in 1978 (20) and in 1979 (29).

As noted previously by several workers (5, 14, 25), although the evidence for inclusion for *R. aurantiacus* in the genus *Rhodococcus* is not overwhelming, there is no doubt about the distinctness of the taxon. Therefore, this taxon should remain as a member of the genus *Rhodococcus* until its taxonomic status is resolved by nucleic acid studies. That this taxon should be named is especially important in light of a recent report that *R. aurantiacus* is a lung pathogen (27).

All additional morphological, physiological, and biochemical characteristics were determined as previously described (15, 19). Mycolic acid analyses were carried out by the methods of Tomiyasu and Yano (14).

Description of *Rhodococcus sputi* (ex Tsukamura 1978) sp. nov., nom. rev. The following description of *Rhodococcus sputi* (spu'ti. L. gen. n. *sputi* of sputum) is based on previous descriptions (20, 22–25) of 16 strains. Gram-positive, slightly acid-fast short rods or coccoid forms (2 by 0.5 to 0.7 μ m). Mycelium is not formed. Colonies on Ogawa egg medium and on Sauton agar are rough and pinkish. Growth occurs abundantly on Ogawa egg medium after 3 days. Growth occurs at 28 and 37°C, but not at 42°C. On Ogawa egg medium, growth occurs in the presence of 0.2% *para*-aminosalicylate, 0.5 mg of sodium salicylate per ml, 0.25 mg of hydroxylamine hydrochloride per ml, 25 μ g of rifampin per ml, and 5 μ g of ethambutol per ml. On Sauton agar growth occurs in the presence of 0.2% picric acid, but not in the presence of 0.1% sodium nitrite. Tween 80 is not hydrolyzed after 14 days. Catalase positive. Acid phosphatase positive. β -Galactosidase negative. α -Esterase positive and β -esterase negative. Nitrate is reduced to nitrite after 24 h. Urease, nicotinamidase, and pyrazinamidase are produced, but benzamidase, isonicotinamidase, salicylamidase, and succinamidase are not produced. Some strains produce acetamidase and allantoinase. *para*-Aminosalicylate and salicylate are not degraded to catechol. Glutamate, acetamide, and monoethanolamine are utilized as simultaneous nitrogen and carbon sources, but serine, D-glucosamine hydrochloride, benzamide, and trimethylenediamine are not. Acetate, citrate, succinate, L-malate, pyruvate, fumarate, glucose, fructose, sucrose, ethanol, *n*-propanol, *n*-butanol, and iso-butanol are utilized as sole carbon sources in the presence of ammoniacal nitrogen, but benzoate, malonate, propylene glycol, 1,3-butylene glycol, 1,4-butylene glycol, and 2,3-butylene glycol are not. Acid is formed from glucose, D-mannose, trehalose, mannitol, and sorbitol. Acid is not formed from D-galactose, L-arabinose, D-xylose, L-rhamnose, and inositol. Nitrate but not nitrite is used as a sole nitrogen source in the presence of glycerol as a carbon source.

The results of gas chromatographic and mass spectrometric analyses show that the mycolic acids of *R. sputi* are composed of eight elements having 58, 60, 61, 62, 63, 64, 65,

and 66 carbon atoms and contain two to five double bonds. The carbon atom numbers are centered at 62. These features of the mycolic acids are apparently different from those of other rhodococci except *R. bronchialis* (1, 4, 31). The mycolic acids of *R. sputi* are differentiated from those of *R. bronchialis* by the following two characteristics: the α -unit of the mycolic acids of *R. sputi* is C_{16:0}, whereas that of the mycolic acids of *R. bronchialis* is C_{18:0}; and the average carbon atom number of the mycolic acids of *R. sputi* is two carbon atoms smaller than the average carbon atom number of the mycolic acids of *R. bronchialis*.

Occurs in the sputum of patients with pulmonary disease.

The type strain is strain ATCC 29627.

Characteristics which differentiate *R. sputi* from all other species of the genus *Rhodococcus* are shown in Table 1.

Description of the type strain. Strain ATCC 29627^T (T = type strain) has all the properties listed above for the species. In addition, the type strain does not produce acetamidase and allantoinase. This strain was isolated from the sputum of a human patient.

Description of *Rhodococcus aurantiacus* (ex Tsukamura and Mizuno 1971; Tsukamura 1974) sp. nov., nom. rev. (Synonym, *Gordona aurantiaca* Tsukamura and Mizuno 1971.) The following description of *Rhodococcus aurantiacus* (au. ran. ti'a. cus. L. adj. *aurantiacus* orange colored) is based on 11 strains. Gram-positive, weakly acid-fast rods (2 to 5 by 0.5 μ m). Some strains are strongly acid fast. Mycelium is not formed. Nonsporeforming. Rough, dry, brownish, scotochromogenic colonies are formed on egg media and on Sauton agar when glutamate is substituted for asparagine. Abundant growth occurs after 3 days. Growth occurs at 28 and 37°C but not at 42°C.

Resistant to 20 μ g of 5-fluorouracil per ml and to 5 μ g of mitomycin C per ml in Ogawa egg medium. Susceptible to 5 μ g of bleomycin per ml in Sauton agar.

Resistant to sodium *para*-aminosalicylate (2 mg/ml), sodium salicylate (0.5 mg/ml), *para*-nitrobenzoic acid (0.5 mg/ml), hydroxylamine hydrochloride (0.5 mg/ml), ethambutol (5 μ g/ml), rifampin (25 μ g/ml), isoniazid (10 μ g/ml), and 5% sodium chloride in Ogawa egg medium and in Löwenstein-Jensen medium. Resistant to 0.2% picric acid, 0.1% sodium salicylate, and 0.2% sodium nitrite in Sauton agar.

Niacin is not produced. *para*-Aminosalicylate and salicylate are not degraded to catechol. Arylsulfatase negative (14 days). Catalase is produced. Tween 80 is usually hydrolyzed after 14 days. β -Galactosidase is produced. Acid phosphatase is not produced. Nitrate is not reduced to nitrite after 24 h. Acetamidase, urease, nicotinamidase, pyrazinamidase, and allantoinase are produced, but benzamidase, isonicotinamidase, salicylamidase, and succinamidase are not.

Glutamate, serine, D-glucosamine hydrochloride, acetamide, and monoethanolamine are utilized as simultaneous nitrogen and carbon sources, but benzamide and trimethylenediamine are not. Acetate, citrate, succinate, L-malate, pyruvate, and fumarate are utilized as sole carbon sources in the presence of ammoniacal nitrogen, but benzoate and malonate are not. Glucose, fructose, sucrose, mannose, galactose, trehalose, inositol, mannitol, and sorbitol are utilized as sole carbon sources in the presence of ammoniacal nitrogen, but L-arabinose, D-xylose, and L-rhamnose are not. Acid is formed from glucose, D-mannose, D-galactose, trehalose, inositol, mannitol, and sorbitol. Acid is not formed from L-arabinose, D-xylose, and L-rhamnose. Ethanol, *n*-propanol, propylene glycol, *n*-butanol, iso-butanol, and 2,3-butylene glycol are utilized as sole carbon

TABLE 1. Characters useful for differentiating species of the genus *Rhodococcus*

Character ^a	Reaction of:															
	<i>R. rhodochrous</i> (n = 7) ^b	<i>R. bronchialis</i> (n = 33)	<i>R. rubroperitinctus</i> (n = 10)	<i>R. terrae</i> (n = 20)	<i>R. lentifragmentus</i> (<i>R. ruber</i>) (n = 11)	<i>R. spati</i> (n = 16)	<i>R. aurantiacus</i> (n = 11)	<i>R. erythropolis</i> (n = 3)	<i>R. equi</i> (n = 6)	<i>R. rhodnii</i> (n = 2)	<i>R. coprophilus</i> (n = 4)	<i>R. aichiensis</i> (n = 5)	<i>R. chubuensis</i> (n = 3)	<i>R. obuensis</i> (n = 7)	<i>R. luteus</i> ^c	<i>R. maris</i> ^c
Rough colonies	100 ^d	100	100	100	100	100	100	100	0	100	100	100	100	100	—	—
Growth after 3 days	100	100	100	100	100	100	100	100	100	100	0	100	100	100	+	—
Growth at 42°C	0	8	0	0	0	0	0	0	0	0	0	100	0	0	—	—
Resistance to NH ₂ OH·HCl (500 µg/ml) ^f	0	8	20	0	36	0	100	33	83	0	0	0	0	0	—	—
Tolerance to 0.2% picric acid ^g	100	100	100	100	100	100	100	100	83	100	0	100	100	100	+	+
Tolerance to 0.1% NaNO ₂ ^g	100	48	100	100	100	0	100	0	100	100	0	80	0	71	+	+
Tolerance to 0.1% salicylate ^g	86	21	20	0	91	0	91	0	100	50	0	80	33	71	+	—
Resistance to ethambutol (5 µg/ml) ^f	100	100	100	100	45	100	100	0	83	100	0	100	100	100	—	—
Resistance to rifampin (25 µg/ml) ^f	100	58	100	100	0	100	100	0	67	0	0	60	100	100	—	—
Tween hydrolysis (14 days)	0	3	0	0	27	69	91	67	33	0	50	100	100	0	—	—
α-Esterase	0	0	30	100	0	38	0	NT ^h	50	0	0	0	100	0	—	—
β-Esterase	100	97	90	100	0	13	45	NT	50	0	0	0	100	0	+	+
β-Galactosidase	0	3	0	0	9	0	91	100	67	50	0	0	0	0	—	—
Acid phosphatase	14	94	0	0	9	88	9	100	67	0	0	100	100	100	—	—
Acetamidase	14	100	20	0	91	69	91	100	100	100	0	80	33	100	—	—
Urease	43	97	100	100	0	100	100	100	33	100	75	100	33	100	+	+
Nicotinamidase	0	94	40	80	73	100	82	100	0	100	0	100	0	100	+	+
Allantoinase	0	6	80	95	0	69	100	67	0	0	25	0	33	14	+	—
Serine used as N and C sources	0	12	0	0	0	13	91	67	0	0	0	0	0	0	—	—
Glucosamine used as N and C sources	100	18	90	90	55	13	91	100	0	0	0	20	0	14	+	—
Acetamide used as N and C sources	0	97	20	0	100	88	100	67	50	0	0	100	67	0	—	—
Monothanolamine used as N and C sources	0	6	80	95	82	88	100	100	0	0	0	100	33	14	—	—
Benzoate used as C source	57	0	60	0	82	0	18	0	0	0	0	0	0	0	—	—
Fumarate used as C source	100	100	100	100	100	88	100	100	67	100	0	60	100	0	+	—
Sucrose used as C source	100	100	100	100	91	100	100	100	0	100	0	100	100	100	+	+
Propylene glycol used as C source	100	48	60	0	91	6	82	67	67	0	0	0	0	0	+	—

iso-Butanol used as C source	100	36	100	100	100	100	69	91	67	83	50	0	100	0	0	+	-
Mannose used as C source	100	100	100	100	100	100	100	100	100	0	50	0	100	100	57	+	-
Galactose used as C source	0	0	0	0	0	0	0	91	0	0	0	0	0	0	0	-	-
Rhamnose used as C source	0	0	0	100	0	100	0	0	0	0	0	0	0	0	0	-	-
Trehalose used as C source	0	100	100	100	27	100	100	100	100	0	0	0	80	100	43	-	-
Inositol used as C source	0	100	0	0	0	0	0	91	100	0	0	0	0	0	0	-	-
Mannitol used as C source	0	42	100	100	100	100	100	100	100	0	100	0	0	100	71	+	-
Sorbitol used as C source	0	0	100	100	100	100	100	100	100	0	100	0	0	100	57	+	-
Nitrate reduction (24 h)	100	91	100	100	91	44	91	9	0	50	50	100	60	67	57	-	+

^a Tests for utilization of carbohydrates as carbon sources were performed in the presence of ammoniacal nitrogen.

^b Number of strains tested.

^c Only the type strains of *R. luteus* (strain VKM A594 [= IMV 385]) and *R. maris* (strain VKM A593 [= IMV 195]) were tested.

^d The values are the percentages of strains showing positive reactions.

^e +, Positive reaction; -, negative reaction.

^f Tested in Ogawa egg medium.

^g Tested in a modified Sauton agar medium, in which sodium glutamate was substituted for asparagine.

^h NT, Not tested.

sources in the presence of ammoniacal nitrogen, but 1,3-butylene glycol and 1,4-butylene glycol are not. Serine, acetamide, urea, pyrazinamide, nicotinamide, and nitrate are utilized as sole nitrogen sources in the presence of glycerol as a carbon source. Nitrite is utilized by some strains.

On silica gel thin-layer chromatography plates, two adjacent spots, corresponding to mycolic acids possessing different straight-chain and alpha-alkyl branch structures, are detected. The lower spot contains monoenoic, dienoic, trienoic, and tetraenoic mycolic acids having 62 to 74 carbon atoms. The upper spot contains dienoic to hexanoic (heptanoic) mycolic acids having 64 to 78 carbon atoms. Based on the numbers of carbon atoms in the mycolic acids, *R. aurantiacus* appears to be closely related to mycobacteria, and based on the number of double bonds, it appears to be closely related to nocardiae and rhodococci. The details have been described by Tomiyasu and Yano (14). The mycolic acids of this organism have also been studied by Goodfellow et al. (5).

Characteristics which differentiate this species from other species of the genus *Rhodococcus* are shown in Table 1.

Occurs in the sputum of human patients with lung disease. This species may be a lung pathogen.

The type strain is strain ATCC 25938.

Description of the type strain. Strain ATCC 25938^T has all of the properties given above for the species except the following. This strain is almost strongly acid fast. It grows at 42°C but not at 45°C. It is susceptible to 0.5 mg of hydroxylamine per ml and resistant to 0.25 mg of hydroxylamine per ml in Ogawa egg medium and Löwenstein-Jensen medium. Tween 80 is not hydrolyzed after 14 days. Nitrite is utilized as a sole nitrogen source. Strain ATCC 25938^T was isolated from the sputum of a patient suffering from lung disease.

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