Rhodococcus chubuensis Tsukamura 1982 Is a Later Subjective Synonym of *Gordona sputi* (Tsukamura 1978) Stackebrandt 1989 comb. nov.

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The genus *Gordona* was proposed by Tsukamura in 1971 (M. Tsukamura, *J. Gen. Microbiol.* 68:15–24, 1971) and was revived in 1988 by Stackebrandt et al. (E. Stackebrandt, J. Smida, and M. D. Collins, *J. Gen. Appl. Microbiol.* 34:341–348, 1988) to accommodate *Rhodococcus* species containing mycolic acids with 48 to 66 carbon atoms and MK-9(H₂) as the predominant menaquinone type. On the basis of mycolic acid composition as determined by high-performance liquid chromatography, DNA-DNA similarity data (S1 nuclease procedure), and the rRNA gene restriction patterns of the type strains, we propose that *Rhodococcus chubuensis* Tsukamura 1982 is a subjective synonym of *Gordona sputi* (Tsukamura 1978) Stackebrandt 1989 comb. nov. We found that *R. chubuensis* type strain ATCC 33609, and *G. sputi* type strain ATCC 29627 exhibit identical mycolic acid profiles, 97% DNA relatedness (difference in thermal denaturation midpoints, 1.0°C), and similar ribotypes after *Pvu*II endonuclease digestion. Thus, as defined by the type strains and considering the priority of the name *G. sputi*, these two species should be regarded as a single species named *Gordona sputi*; an emended description of this taxon is given.

The genus *Gordona*, with the species *Gordona rubra*, *Gordona terrae*, and *Gordona bronchialis*, was proposed by Tsukamura in 1971 for coryneform bacteria isolated from sputa of patients with pulmonary disease and from soil (17). This genus was distinguished from rapidly growing mycobacteria mainly on the basis of its slight acid fastness and the absence of arylsulfatase and from nocardiae on the basis of a positive nitrate reduction reaction and the absence of mycelium. In 1977 Goodfellow and Alderson delineated by numerical taxonomy the genus *Rhodococcus* for strains previously placed in the species *Mycobacterium rhodochrous* and the “*rhodochrous* complex,” including members of the genus *Gordona* (5). However, additional studies revealed that the genus *Rhodococcus* was heterogeneous. The three *Rhodococcus* species originally assigned to the genus *Gordona* were found to contain mycolic acids with 48 to 66 carbon atoms and MK-9(H₂) as the predominant menaquinone type, which differentiated these organisms from the other *Rhodococcus* species studied (4, 15). In 1988 Stackebrandt et al. revived the genus *Gordona*, with the three previously described species, on the basis of 16S rRNA homology results and, in addition, included the species *Rhodococcus sputi* in this revived genus (13, 14). Addenda concerning the species *Gordona sputi* were published by Tsukamura in 1991; these addenda contained mycolic acid and menaquinone composition data for this organism, which confirmed its status (20).

Rhodococcus chubuensis was described in 1982 by Tsukamura in a numerical analysis study of the taxonomy of nocardiae and rhodococci isolated from human specimens (19). This taxon was included in the genus *Rhodococcus* and was differentiated from other species solely on the basis of morphological and biochemical properties. Although no chemotaxonomic or genomic studies were performed to verify its taxonomical

status, *R. chubuensis* was subsequently validated as a new species in 1983, with strain E 6324 (= ATCC 33609) as the type strain (8, 10). Because of its questionable status, in *Bergey's Manual of Systematic Bacteriology* Goodfellow considered *R. chubuensis* a species incertae sedis of the genus *Rhodococcus* (4). Moreover, in 1986 Tomiyasu and Yano (15) found by using gas-liquid chromatography mycolic acids with 55 to 65 carbon atoms in a strain of *R. chubuensis*; this is a characteristic of the genus *Gordona*. Nevertheless, in 1992 *R. chubuensis* was included in an emended version of the Approved Lists of Bacterial Names (11).

This situation led us to reinvestigate the taxonomic position of this species. In this study we determined that *R. chubuensis* and *G. sputi* are synonyms on the basis of mycolic acid composition data, DNA-DNA similarity data, and the rRNA gene restriction patterns of the type strains.

MATERIALS AND METHODS

Bacterial strains. For *R. chubuensis*, we used only type strain ATCC 33609 (provided by the American Type Culture Collection) since very few *R. chubuensis* strains are available. Reference strains *G. sputi* ATCC 29627^T (T = type strain), *G. bronchialis* ATCC 25592^T, and *Rhodococcus rhodochrous* ATCC 13808^T were also obtained directly from the American Type Culture Collection, and reference strains *Gordona rubropertincta* ATCC 25593, *G. terrae* ATCC 25594^T, and *G. sputi* 1834-89 were kindly provided by P. Boiron, Institut Pasteur, Paris, France.

Growth conditions and biochemical tests. Bacteria were grown aerobically at 30 and 37°C on Mueller-Hinton agar and on Coletso medium. Utilization of citrate, benzoate, inositol, mannitol, L-rhamnose, sorbitol, 2,3-butanediol, monoethanolamine, and *n*-butanol was determined by using media prepared as described by Tsukamura (16). Urease production was tested in Christensen's medium, and nitrate reductase production was tested by using a brain heart broth supplemented with 2 g of NaNO₃ per liter. Enzymatic activities were examined by using

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FIG. 1. HPLC patterns of *p*-bromophenacyl esters of mycolic acids from *R. rhodochrous* ATCC 13808^T (A), *G. sputi* ATCC 29627^T (B), *G. bronchialis* ATCC 25592^T (C), and *R. chubuensis* ATCC 33609^T (D).

the API-ZYM system according to the instructions of the manufacturer (bioMérieux, Marcy l'Etoile, France).

Mycolic acid analysis. Reversed-phase high-performance liquid chromatography (HPLC) was performed to determine mycolic acid contents. Saponification, derivatization, and chromatographic separation were carried out by using the procedure described for the analysis of *rhodococcus* species in a previous report (3).

DNA-DNA hybridization. DNA was extracted and purified as described previously (12). DNA from *R. chubuensis* ATCC 33609^T was labeled by nick translation with [³H]dCTP (Amersham International, Amersham, United Kingdom), using a

commercial kit (Gibco Bethesda Research Laboratories). Duplicate hybridization experiments were performed at 65°C for 16 h in 0.42 M NaCl by the S1 nuclease-trichloroacetic acid method (7). The denaturation temperature at which 50% of the reassociated DNA became hydrolyzable by S1 nuclease was determined in 0.2 M NaCl by the method of Crosa et al. (2). ΔT_m was the difference between the denaturation temperature of a homoduplex (in a homologous reaction) and the denaturation temperature of a heteroduplex (in a heterologous reaction).

Ribotyping. DNA was digested with restriction endonuclease *Pvu*II. DNA fragments were separated by agarose gel

TABLE 1. Levels of DNA relatedness between *R. chubuensis* ATCC 33609^T and *Gordona* reference strains

Source of unlabeled DNA	% Relative reassociation with strain ATCC 33609 ^T at 65°C
<i>R. chubuensis</i> ATCC 33609 ^T	100
<i>G. sputi</i> ATCC 29627 ^T	97 (1.0) ^a
<i>G. sputi</i> 1834-89.....	100 (1.0)
<i>G. bronchialis</i> ATCC 25592 ^T	7
<i>G. rubropertincta</i> ATCC 25593.....	7
<i>G. terrae</i> ATCC 25594 ^T	9

^a The values in parentheses are ΔT_m values (in degrees Celsius).

electrophoresis, transferred to a nylon membrane, and hybridized with 5'-³²P-labeled *Escherichia coli* 16S + 23S rRNA for 16 h at 60°C as described by Grimont and Grimont (6).

RESULTS AND DISCUSSION

Mycolic acid analysis. The results of an analysis of mycolic acid composition by the HPLC method, which has been determined to be a reliable tool for the identification of actinomycetes (1), confirmed the previous findings (15) concerning the relationship of *R. chubuensis* and *Gordona* species. Figure 1 shows the similar patterns of the *p*-bromophenacyl esters of the mycolic acids of *R. chubuensis* ATCC 33609^T and the type strains of *G. sputi* and *G. bronchialis*; these patterns differentiated these organisms from the type strain of the type species of the genus *Rhodococcus*, *R. rhodochrous*, whose chromatography profile contained mycolic acids with shorter retention times.

DNA-DNA similarities. As shown in Table 1, DNA-DNA hybridization experiments revealed that there was a high level of genomic similarity between the type strains of *R. chubuensis* and *G. sputi*. We used the generally accepted criteria for defining a genomic species (23), and the level of DNA-DNA relatedness (97%) and the difference in the thermal denaturation midpoints (ΔT_m , 1°C) which we determined in this study led us to consider the type strains of *R. chubuensis* and *G. sputi* members of a single species. No significant levels of DNA similarity with the other reference strains of *Gordona* species were observed.

Ribotyping. Ribotyping data confirmed the DNA-DNA results; the patterns for the type strains of *R. chubuensis* and *G. sputi* were similar when endonuclease *Pvu*II digestion was used (Fig. 2). In a previous study, Lasker et al. (9) characterized *Rhodococcus* and *Gordona* species by performing an rRNA gene restriction pattern analysis of the type strains. The ribotypes obtained for *R. chubuensis* ATCC 33609^T and *G. sputi* ATCC 29627^T by these authors with the same restriction enzyme apparently are not different from the patterns obtained in this study, and this observation allowed us to rule out any possible mislabeling or contamination in our laboratory. Thus, as defined by the type strain, we confirmed the taxonomic similarity between *R. chubuensis* and *G. sputi*.

Phenotypic characterization. The morphologic characteristics of *R. chubuensis* ATCC 33609^T were similar to the characteristics given in previous descriptions (19). This organism produced dry, raised, beige colonies without mycelium, and the colonies became salmon colored after several days of culture. Strain ATCC 33609^T was gram-positive and acid fast with a coryneform aspect and did not exhibit extensive branching. These characteristics are in agreement with those of *Gordona* species (14, 17). We also found that nitrate reductase

FIG. 2. rRNA gene restriction patterns after cleavage by *Pvu*II. Lanes 1 to 3, *G. bronchialis* ATCC 25592^T, 1828-89, and 1829-89, respectively; lane 4, *G. rubropertincta* ATCC 25593; lanes 5 and 6, *G. terrae* ATCC 25594^T and 1837-89 respectively; lanes 7 and 8, *G. sputi* ATCC 29627^T and 1834-89 respectively; lane 9, *R. chubuensis* ATCC 33609^T; lane 10, *Bst*EII fragments of lambda DNA (sizes [in kilobases] are indicated on the right).

and urease activities are present in strain ATCC 33609^T and β -galactosidase activity is not, which is a characteristic of the genus *Gordona* that differentiates it from the genus *Nocardia* (19). This strain can use citrate, *n*-butanol, monoethanolamine, mannitol, sorbitol, or 2,3-butanediol but not benzoate, inositol, or L-rhamnose as a sole carbon source. Among the biochemical data reported in the original description of *R. chubuensis* (19), differentiation of *R. chubuensis* from *G. sputi* was based primarily on the presence of β -esterase activity and Tween 80 hydrolysis in *R. chubuensis* and the ability of *R. sputi* to grow with isobutanol as a carbon source. Additional studies (21, 22) have modified these data and have revealed that there are no major differences between the two species in the results of these tests. Furthermore, in 1988 Zakrewska-Czerwinska et al. (24) found on the basis of DNA similarity data for the type strains that *Rhodococcus obuensis* could be considered a synonym of *G. sputi*. On the basis of these data and our results, below we propose an emended description of *G. sputi* (Tsukamura 1978) Stackebrandt 1989 comb. nov. Table 2 shows the major biochemical characteristics that differentiate *G. sputi* from the other *Gordona* species.

Emended description of *Gordona sputi* (Tsukamura 1978) Stackebrandt 1989 comb. nov. The salient characteristics of *G. sputi*, based on the descriptions of *R. sputi* of Tsukamura and Yano (22) and Tsukamura (18), the data of Zakrewska-Czerwinska et al. (24), and our observations, are as follows. Gram-positive, slightly acid-fast short rods or coccoid forms. Does not form spores. Mycelium is not formed. Strict aerobes. Growth occurs at 28 and 37°C, but not at 42°C. Colonies on Sauton agar are rough and pinkish. On Mueller-Hinton medium, dry, raised, beige colonies become salmon colored after several days of culture. Catalase positive and oxidase negative.

TABLE 2. Characteristics used to differentiate *G. sputi* from other *Gordona* species

Species	Utilization of ^a :						
	Benzoate	Inositol	Mannitol	L-Rhamnose	Sorbitol	n-Butanol	Monoethanolamine
<i>G. sputi</i>	—	—	+	—	+	+	+
<i>G. bronchialis</i>	d	+	—	—	—	+	—
<i>G. terrae</i>	+	—	+	+	+	+	+
<i>G. rubropertincta</i>	+	—	+	—	+	—	+

^a Data from references 21 and 22 and our observations. —, 10% or less of the strains are positive; +, 90% or more of the strains are positive; d, 11 to 89% of the strains are positive. Data were obtained by using the procedure described by Tsukamura (16). The data indicate the utilization of benzoate, inositol, mannitol, L-rhamnose, sorbitol, and n-butanol as sole carbon sources and the utilization of monoethanolamine as a simultaneous nitrogen and carbon source.

Acid phosphatase, nitrate reductase, urease, and pyrazinamidase are produced, but β -galactosidase, benzamidase, isonicotinamidase, salicylamidase, and succinamidase are not produced. Production of α -esterase, β -esterase, nicotinamidase, acetamidase, and allantoinase and hydrolysis of Tween 80 are variable. Glutamate and monoethanolamine are utilized by most strains as simultaneous nitrogen and carbon sources, but serine, D-glucosamine hydrochloride, benzamide, and trimethylenediamine are not utilized.

Acetate, citrate, succinate, L-malate, pyruvate, glucose, fructose, sucrose, mannitol, sorbitol, ethanol, n-propanol, and n-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, inositol, and L-rhamnose are not utilized; utilization of fumarate, utilization of 2,3-butylene glycol, and utilization of isobutanol are variable. Acid is formed from glucose, D-mannose, trehalose, mannitol, and sorbitol but not 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 this organism has long-chain mycolic acids (numbers of carbon atoms, 56, 58, 60, 62, 64, and 66). The numbers of carbon atoms in the α unit are 16 and 18, the numbers of carbon atoms in the β unit are 40 to 48, and the numbers of double bonds in the mycolic acids are 2 to 6. The predominant menaquinone type is MK-9(H₂), and there is a significant amount of MK-8(H₂), (9 and 8 are the numbers of isoprene units in the side chain and 2 is the number of hydrogen atoms saturating the side chain). The cell wall peptidoglycan contains major amounts of *meso*-diaminopimelic acid, arabinose, and galactose.

The type strain is strain ATCC 29627, which was isolated from the sputum of a human patient. Strain ATCC 29627^T has all of the properties described above for the species. In addition, the type strain does not produce acetamidase and allantoinase. The G+C content of the DNA is 65.2 mol%, as determined by an HPLC procedure.

ACKNOWLEDGMENTS

We are grateful to P. Boiron (Institut Pasteur, Paris, France) for kindly providing reference strains and for helpful discussions. We thank C. Gallion and B. Muller for expert technical assistance.

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