

Biology of the Metabolically Diverse Genus *Gordonia*

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The actinomycete genus *Gordonia* has attracted much interest in recent years for a variety of reasons. Most species were isolated due to their abilities to degrade xenobiotics, environmental pollutants, or otherwise slowly biodegradable natural polymers as well as to transform or synthesize possibly useful compounds. The variety of chemical compounds being transformed, biodegraded, and synthesized by gordoniae makes these bacteria potentially useful for environmental and industrial biotechnology. However, because some gordoniae are opportunistic pathogens, their application in the environment may be restricted in some cases. Valuable phenotypic features of *Gordonia* species can be genetically transferred to other microorganisms, as has been demonstrated by Park et al. (76) and Bröker et al. (12). Recent studies have revealed improved cloning vectors allowing the transfer of genes between different *Gordonia* species or the transfer of foreign genes from *Escherichia coli* to *Gordonia* spp. and the expression of these genes (3, 12). Current research also focuses on several species that are known to cause infections, especially in humans. *Gordonia* species have been isolated from various native biotopes such as soil or mangrove rhizosphere, from extensively industrially influenced habitats such as oil-producing wells or hydrocarbon-contaminated soil, from artificial sources such as wastewater treatment bioreactors or biofilters, and from diseased humans. The genus *Gordonia* belongs phylogenetically to the suborder *Corynebacterineae*, the mycolic acid group within the order *Actinomycetales* (86), and its classification has changed drastically in recent years, with several species being reclassified and many novel species being described.

This review provides an overview of recent research on *Gordonia* species and summarizes the importance of this emerging genus in industrial and environmental biotechnology and its role as a pathogen of humans. Since the introduction of the correct name in 1997 (see below), the number of publications per annum listed by the Science Citation Index if “*Gordonia*” is entered as the key word and only studies on prokaryotes are considered has increased steadily: from 3 in 1998 to 7 in 1999, 10 in 2000, 15 in 2001, 13 in 2002, and 18 in 2003 (until November). In addition, as of November 2003, more than 40 patents relating to *Gordonia* species have been registered, indicating their increasingly recognized commercial potential.

TAXONOMY OF THE GENUS *GORDONIA*

In 1971, Tsukamura proposed *Gordona* as a new genus for coryneform bacteria isolated from sputa of patients with pul-

monary disease or from soil (91). The name for this novel genus was chosen to pay tribute to the American bacteriologist Ruth E. Gordon. Members of this genus are distinguished from fast-growing mycobacteria by their slight acid fastness and the absence of arylsulfatase, and from the genus *Nocardia* by their ability to reduce nitrate and the absence of a mycelium. Only six years later, Goodfellow and Alderson discarded this taxon and transferred *Mycobacterium rhodochrous* and the “*rhodochrous* complex,” including representatives of the genus *Gordona*, to the genus *Rhodococcus* as “a home for the *rhodochrous* complex” (34). However, studies of the mycolic acid (high-molecular-weight α -branched 3-hydroxy fatty acid) and menaquinone composition revealed heterogeneous variations within the genus *Rhodococcus* (33, 90): while *Rhodococcus* spp. usually contain mycolic acids with 34 to 52 carbon atoms and menaquinones with 8 isoprene units and 2 hydrogen atoms added to the isoprene side chain double bonds [MK-8(H₂)] as the major form of menaquinone, three *Rhodococcus* species originally assigned to the genus *Gordona* (*Gordona bronchialis*, *Gordona rubripertincta* [formerly *Gordona rubra*], and *Gordona terrae*) were found to contain mycolic acids of 48 to 66 carbon atoms and dihydrogenated menaquinones with 9 isoprene units [MK-9(H₂)] as the predominant menaquinone. Additional analysis of 16S rRNA similarities led to a revival of the genus *Gordona* by Stackebrandt et al. (87) with the three species mentioned above and, additionally, *Rhodococcus sputi* (92) as *Gordona sputi*. After reclassification of *Rhodococcus aichiensis* (93) and *Nocardia amarae* (59) to the genus *Gordona* as *Gordona aichiensis* and *Gordona amarae* (50), respectively, this taxon became a well-defined genus within the order *Actinomycetales*. In 1997, the etymologically correct name *Gordonia* instead of *Gordona* was proposed by Stackebrandt et al. (86). According to their newly proposed hierarchic classification system for the actinomycete line, *Gordonia* is the type genus of *Gordoniaceae* (the *Gordonia* family) within the suborder *Corynebacterineae*. This suborder includes also the families *Corynebacteriaceae*, *Dietziaceae*, *Mycobacteriaceae*, *Nocardiaceae*, *Tsukamurellaceae*, and *Williamsiaceae*. Since the report of *Gordonia hydrophobica* as a new species (7), the number of validly described members of this genus has increased considerably. At present, the genus *Gordonia* comprises 19 validly published species, and at least 2 further species are in the process of classification (Table 1). Meanwhile, gordoniae have been shown to be ubiquitously distributed in nature.

Interestingly, the taxon *Gordonia* also exists in the plant kingdom. There, the genus *Gordonia* comprises evergreen trees, and it belongs to the family *Theaceae* (27). Because the bacterial taxon *Gordonia* was introduced before the revised principle 2 of the “Code of Nomenclature of Prokaryotes” was

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TABLE 1. Overview of described *Gordonia* species and their relevant characteristics

Type strain	Characteristic or source	Reference for type strain
<i>G. aichiensis</i> DSM 43978 ^T	Clinical isolate from sputum	93
<i>G. alkanivorans</i> DSM 44369 ^T	Alkane-degrading bacterium	55
<i>G. amarae</i> DSM 43392 ^T	Foam-forming bacterium	59
<i>G. amicalis</i> DSM 44461 ^T	Dibenzothiophene-desulfurizing bacterium	49
<i>G. bronchialis</i> DSM 43247 ^T	Isolated from sputum of a woman with cavitary pulmonary tuberculosis	91
<i>G. desulfuricans</i> DSM 44462 ^T	Benzothiophene-desulfurizing bacterium	48
<i>G. hirsuta</i> DSM 44140 ^T	Isolated from a biofilter of an animal-rendering plant	51
<i>G. hydrophobica</i> DSM 44015 ^T	Isolated from a biofilter of an animal-rendering plant	7
<i>G. jacobaea</i> MV-1 ^a	Canthaxanthin-containing bacterium	22
<i>G. namibiensis</i> DSM 44568 ^T	Nitrile-metabolizing bacterium	11
<i>G. nitida</i> DSM 44499 ^T	3-Ethyl- and 3-methylpyridine-degrading bacterium	95
<i>G. paraffinivorans</i> DSM 44604 ^T	Hydrocarbon-degrading bacterium	94
<i>G. polyisoprenivorans</i> DSM 44302 ^T	<i>cis</i> -1,4-Polyisoprene-degrading bacterium	62
<i>G. rhizosphaera</i> DSM 44383 ^T	Isolated from mangrove rhizosphere	89
<i>G. rubripertincta</i> DSM 43197 ^T	Mycobactin-producing bacterium	91
<i>G. sihwensis</i> DSM 44576 ^T	Nitrate-reducing bacterium	47
<i>G. sinesedis</i> DSM 44455 ^T	Isolated from soil	28, 66
<i>G. spumae</i> T4 ^a	Isolated from activated sludge foam	Sukhoom et al., unpublished data
<i>G. sputi</i> DSM 43896 ^T	Isolated from sputum of a patient with pulmonary disease	92
<i>G. terrae</i> DSM 43249 ^T	Isolated from soil	91
<i>G. westfalica</i> DSM 44215 ^T	<i>cis</i> -1,4-Polyisoprene-degrading bacterium	64

^a Not a validly described species.

published (24), this name can continue to be used for these bacteria.

MORPHOLOGICAL AND CHEMOTAXONOMIC PROPERTIES

Morphological and structural aspects. Members of the genus *Gordonia* are aerobic, catalase-positive, gram-positive to gram-variable, slightly acid-fast, nonmotile, nocardioform actinomycetes. They have an oxidative carbohydrate metabolism and are arylsulfatase negative; susceptibility to lysozyme has been shown. The term “nocardioform” is morphologically descriptive and refers to mycelial growth with fragmentation into rod-shaped or coccoid elements (58). *Gordoniae* do not generate spores. The colony morphology of *Gordonia* species varies from slimy, smooth, and glossy to irregular and rough; it may even differ within one species depending on the medium used for growth (64). Some strains, such as *Gordonia alkanivorans* DSM 44369^T and *Gordonia westfalica* DSM 44215^T, are also able to form both smooth and rough colonies (55, 64). Usually, cultures with smooth phenotypes can generate rough colonies; this alteration seems to be irreversible and could be due to mutations in genes encoding methyltransferases involved in glycopeptidolipid synthesis, as described for *Mycobacterium smegmatis* (78). The importance of glycosylated peptidolipids for colony morphology has also been demonstrated for *G. hydrophobica* (69). The colors of the colonies cover a broad range, including white, yellow, tannish, orange, red, and pink (94). The cell wall belongs to wall chemotype IV sensu Lechevalier and Lechevalier; the peptidoglycan is of the A1 γ type, containing *meso*-diaminopimelic acid as the only diamino acid and muramic acid with *N*-glycolyl residues (2). The major cell wall sugars are arabinose and galactose. Characteristically, mycolic acids with 44 to 66 carbon atoms and major portions of straight-chain, saturated, monounsaturated, and 10-methyl (tuberculostearic) cellular fatty acids are formed (8). Polar lipids

are typically composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. Cells contain MK-9(H₂) as the predominant isoprenologue. Only in some species can traces of MK-8(H₂) be found. The cell wall composition characteristics and cellular chemotaxonomic properties mentioned above separate *Gordonia* clearly from related genera such as *Mycobacterium*, *Rhodococcus*, and *Skermania* (Table 2).

Taxonomic aspects related to DNA and RNA sequence data. The G+C content of *Gordonia* DNA ranges from 63 to 69 mol% (Table 2), and intragenetic DNA-DNA relatednesses ranges from 3.7% (shared by *Gordonia nitida* and *G. amarae*) (95) to 52% (shared by *G. alkanivorans* and *G. rubripertincta*) (55). The 16S ribosomal DNA (rDNA) sequences of *Gordonia* species reveal similarities ranging from 94.8% (between *G. amarae* and *Gordonia sihwensis*) to 99.9% (between *G. alkanivorans* and *G. nitida*). Phylogenetic trees based on sequence alignments with species of other genera of the suborder *Corynebacterineae* show the distinctly defined inter- and intragenetic phylogenetic positions of *Gordonia* species (Fig. 1). The deviations in the 16S rDNA sequences of different *Gordonia* species occur mostly in two hypervariable regions between nucleotide positions 136 and 229 and between nucleotides 996 and 1028 (according to the numbering of the *E. coli* K-12 16S rDNA sequence) (Fig. 2). All species differ from each other in these regions except *G. alkanivorans* and *G. nitida*. The latter were published individually as novel species at the same time. DNA-DNA-hybridization experiments must still be carried out to confirm the phylogenetic relationship between these species.

PATHOGENIC GORDONIA SPECIES

Most *Gordonia* species have been isolated from environmental sources; however, a few are also sporadically associated with human infections (Table 3). In almost all cases, patients

TABLE 2. Chemotaxonomic markers of genera of the suborder *Corynebacterineae*

Family	Genus	Acyl type ^a	Major menaquinone	PE ^b	Fatty acid compositions ^c	Mycolate size (no. of carbons)	Pyrolysis esters of mycolates ^d (no. of carbons)	G+C content (mol%)
<i>Gordoniaceae</i>	<i>Gordonia</i>	G	MK-9(H ₂)	+	S, U, T	48–66	16–18	63–69
	<i>Skermania</i>	G	MK-8(H ₄ ω-cyc)	+	S, U, T	58–64	16–20	68
<i>Corynebacteriaceae</i>	<i>Corynebacterium</i>	A	MK-8(H ₂)	– ^e	S, U ^f	22–36	8–18	51–67
	<i>Turicella</i>	ND	MK-10, MK-11	ND	S, U, T	– ^h	– ^h	65–72
<i>Dietziaceae</i>	<i>Dietzia</i>	A	MK-8(H ₂)	–	S, U, T	34–38	ND	73
<i>Mycobacteriaceae</i>	<i>Mycobacterium</i>	G	MK-9(H ₂)	+	S, U, T	70–90	22–26	70–72
<i>Nocardiaceae</i>	<i>Nocardia</i>	G	MK-8(H ₄ ω-cyc)	+	S, U, T	50–62	12–18	64–72
	<i>Rhodococcus</i>	G	MK-8(H ₂)	+	S, U, T	34–54	12–16	63–73
<i>Tsukamurellaceae</i>	<i>Tsukamurella</i>	G	MK-9	+	S, U, T	64–78	20:1 ^g	67–68
<i>Williamsiaceae</i>	<i>Williamsia</i>	G	MK-9(H ₂)	+	S, U, T	50–56	ND	64–65

^a G, glycolated muramic acid; A, acetylated muramic acid; ND, not determined.

^b PE, phosphatidylethanolamine; +, present; –, absent.

^c S, saturated; U, unsaturated; T, tuberculostearic acid.

^d Fatty acid methyl esters released by pyrolysis of mycolic acid methyl esters.

^e Present in *Corynebacterium bovis* and *Corynebacterium urealyticum*.

^f Tuberculostearic acid present in *Corynebacterium ammoniagenes*, *C. bovis*, *C. minutissimum*, *C. urealyticum*, and *C. variabile*.

^g 20:1, eicosanoic acid.

^h Mycolic acid free.

were immunosuppressed after underlying diseases, and infections by *Gordonia* species occurred only secondarily. Altogether, approximately 20 case reports of gordonial infections can be found in the literature; most of these infections were caused by *G. bronchialis* and were associated with sternal wounds resulting from surgery (Table 3). A few infections also occurred after coronary artery bypass surgery and were associated with heart-lung machines or applications of central venous or Hickman catheters (Table 3). Two additional infections with *Gordonia* spp. have been reported in the literature; however, these isolates were not identified to species level (61). Interestingly, one of these reports described an infection by a *Gordonia* species taxonomically closely related to *G. sputi*. The pattern of its *p*-bromophenacyl esters of mycolic acids was similar to that of *G. sputi* DSM 43896^T (also classified as ATCC 29627), and several biochemical tests revealed characteristics of *Gordonia* spp., especially those of *G. bronchialis*. The first 450 bp at the 5' end of the 16S rDNA totally matched those of *G. sputi*. Surprisingly, DNA-DNA relatedness to *G. sputi*, as analyzed by the stringent nuclease S1 method, did not meet the genetic criterion of more than 70% similarity to belong to the same species. Consequently, one must suggest that this strain could belong to a novel gordonial species. So far, reports of human gordonial infections are rather rare in comparison to reports on infections caused by other opportunistically pathogenic bacteria belonging to taxonomically related genera such as *Rhodococcus* and *Nocardia*.

CAPABILITIES FOR BIODEGRADATION AND BIOREMEDIATION

Gordoniae are probably important in natural environments and are powerful candidates for bioremediation processes because of their capacity to degrade substituted and nonsubsti-

tuted hydrocarbons, widespread toxic environmental pollutants, other xenobiotics, and natural compounds that are not readily biodegradable. *Gordonia* species may play an important role during wastewater treatment and in biofilters (9). Several *Gordonia* species were isolated due to their capabilities to degrade or modify aliphatic and aromatic hydrocarbons, halogenated aromatic compounds, benzothiophene, nitrile, polyisoprene, xylene, and so forth. Incorporation of the long aliphatic chains of the mycolic acids into the cell wall is associated with hydrophobicity and surface adhesion (8) and may play a role in the degradation of hydrophobic pollutants. Examples of this ability are the adhesive growth of several *Gordonia* strains during the biodegradation of rubber materials (2, 63, 65) and the utilization of hydrophobic hydrocarbons by many species of this genus (55, 94).

Phthalic acid esters. Chatterjee and Dutta (15) described *Gordonia* sp. strain MTCC 4818, isolated from creosote-contaminated soil, which was able to metabolize several phthalic acid esters as sole sources of carbon and energy. Phthalic acid esters exhibit hormonal action due to their binding activity toward the estrogen receptor (71) and can cause proliferation of MCF-7 cells (85). Obviously, this strain was able to hydrolyze both ester bonds of butyl benzyl phthalate and utilized the released benzyl alcohol and butanol for growth. The esterase activity was shown to be induced by butyl benzyl phthalate and/or monobenzyl phthalate, indicating a specificity of the enzyme for this xenobiotic. Surprisingly, *Gordonia* sp. strain MTCC 4818 was unable to metabolize phthalic acid, which therefore accumulated as a dead-end product in the culture broth. Under aerobic conditions, phthalic acid is usually further degraded via protochatechuate (88).

s-Triazine and alkyipyridines. Further examples of xenobiotic pollutants degraded or transformed by members of the

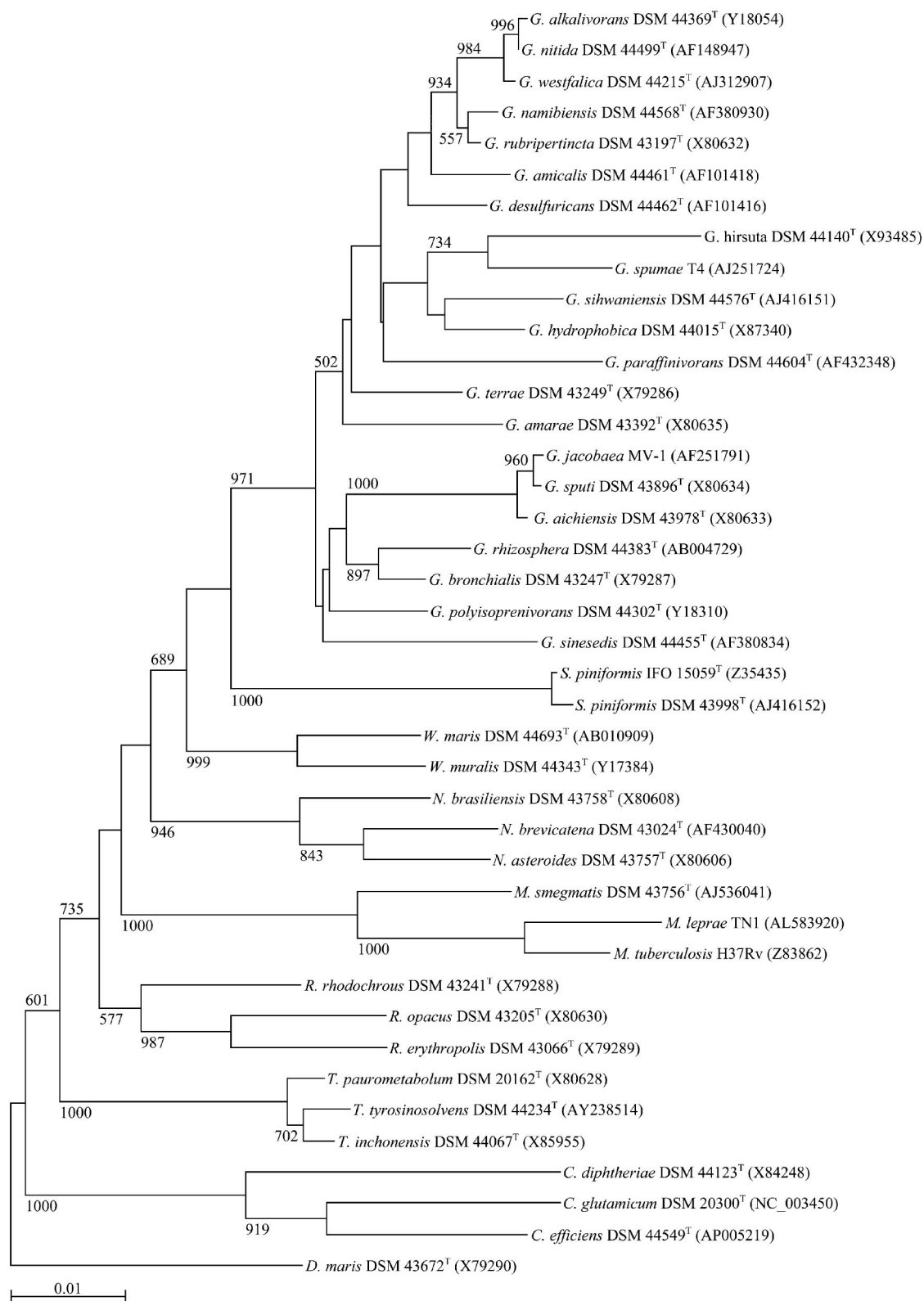


FIG. 1. Neighbor-joining tree (82) based on nearly complete 16S rDNA sequences of gordoniae and representatives of other mycolic acid-containing actinomycetes, including members of the genera *Corynebacterium*, *Dietzia*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Skermania*, *Tsakamurella*, and *Williamsia*. Numbers at nodes indicate the level of bootstrap support based on a neighbor-joining analysis of 1,000 resampled data sets; only values above 50% are given. Bar, 0.01 substitution per nucleotide position.

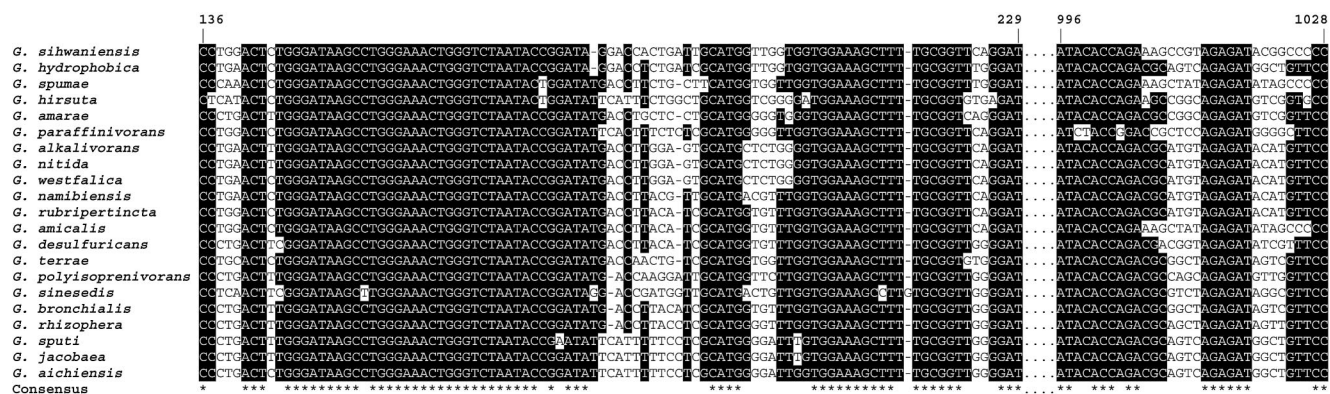


FIG. 2. Comparison of the two major hypervariable regions of the 16S rDNA sequences of the type strains of all *Gordonia* species by multiple alignment. Highlighted nucleotide positions have at least 70% identical nucleotides. Nucleotide positions are given according to the numbering of the *Escherichia coli* K-12 16S rDNA sequence.

gordoniae are heterocyclic compounds such as *s*-triazines and alkylpyridines. The relative persistence of *s*-triazines such as atrazine and simazine has led to increasing concern about environmental contamination by these compounds. So far, only a very few microbial isolates capable of transforming substituted *s*-triazines have been identified. Mulbry purified and characterized a unique inducible *s*-triazine hydrolase from extracts of *G. rubripertincta* DSM 10347 (formerly *Rhodococcus corallinus*) (70); this enzyme was able to dechlorinate atrazine and deethylsimazine as well as to deaminate melamine and related compounds (17, 18). The ability of the triazine hydrolase to dechlorinate *s*-triazine compounds makes this unique enzyme potentially useful for environmental remediation. Alkylpyridines are also toxic environmental pollutants, and despite their occurrence and toxicity, only a few studies have focused on the biodegradation of these compounds. Detailed studies of the degradation pathway were previously performed with *Pseudomonas* sp. strain KM3; however, recent research revealed a novel degradation pathway of 3-ethylpyridine and

3-methylpyridine in the newly isolated and described species *G. nitida* (60, 95).

DBT and biodesulfurization of fuels. The modification of heterocyclic compounds can also be used in the processing of important industrial products such as fossil fuels. Because regulations concerning the sulfur content of motor fuels have become increasingly stringent in an effort to reduce SO_x emissions, sulfur removal by biocatalytic means is often considered a potential alternative to the conventional deep hydrodesulfurization processes used in refineries. The development of convenient and economically feasible microbial processes for the removal of organic sulfur compounds from fossil fuels led to the isolation of *Rhodococcus rhodochrous* ATCC 53968 (44). Dibenzothiophene (DBT) is widely regarded as a model compound that is representative of the aromatic organosulfur fraction of coal and crude oil. DBT is usually transformed to 2-hydroxybiphenyl and inorganic sulfite via the 4S pathway (46, 74). Several *Gordonia* species or strains have been characterized for their ability to desulfurize DBT or benzothiophene (1,

TABLE 3. Infections of humans with gordoniae and underlying conditions

<i>Gordonia</i> species	Type of infection	No. of cases	Underlying condition(s)	Reference
<i>G. bronchialis</i>	Sternal wound	7	Surgery	80
<i>G. polyisoprenivorans</i>	Bacteremia due to Hickman catheter	1	Bone marrow transplantation	45
<i>G. rubripertincta</i>	Lung infection	1	Tuberculosis	37
<i>G. sputi</i>	Mediastinitis	1	Surgery	57
	Bacteremia due to cutaneous lesions	1	Metastatic melanoma; interleukin-2 treatment	81
<i>G. terrae</i>	Brain abscess	1	None	25
		1	Cerebral tumor	26
	Skin infection	1	None	67
	Bacteremia due to central venous catheter	1	Chronic intestinal pseudo-obstruction syndrome	13
<i>Gordonia</i> sp.	Bacteremia due to central venous catheter	1	Breast and ovarian cancer	13
		1	Multiple	61

32, 48, 49). The first representative of the genus *Gordonia* described to be capable of DBT desulfurization was *Gordonia* sp. strain CYKS1 (79). The desulfurization genes (*dszABC*) of this strain and the closely related strain *G. nitida* LSSEJ-1 have been cloned (GenBank accession no. AY396519 and AY293884) and functionally expressed in *E. coli*. The resulting recombinant strains were applied for deep desulfurization of diesel oil (76). Chang et al. (14) described in detail the production of *Gordonia* sp. strain CYKS1 as a desulfurization biocatalyst by a two-stage process and its application for treatment of model and diesel oils. In addition, the phase separation in diesel oil biodesulfurization was optimized by the addition of ethanol as a de-emulsifier to a three-phase (diesel oil-biocatalyst-aqueous phase) emulsion in order to recover the biocatalyst (*Gordonia* sp. strain CYKS1) and the desulfurized oil (16). The enhanced processes allowed a decrease in the sulfur content of diesel oil from 250 to approximately 60 mg liter⁻¹ and should enable the production of ultra-low-sulfur fuel oils by biotechnological means.

Other xenobiotics. Several other poorly accessible carbon sources, such as *t*-butyl ether, methyl *t*-butyl ether, *t*-amyl methyl ether, cyclic alkanes, and polycyclic aromatic hydrocarbons, can be degraded by species of the genus *Gordonia* (38, 42, 43, 52). These catabolic abilities are probably closely related to the production of biosurfactants by microbial cells (41).

Natural and synthetic rubber. The ability to biodegrade natural and synthetic isoprene rubber (*cis*-1,4-polyisoprene) is not widely distributed among bacteria. As described previously, degradation of this polymer can follow one of at least two strategies, and the most potent rubber-degrading bacteria belong to the genus *Gordonia* (2, 65). Until recently, all rubber-degrading bacteria had in common the production of translucent halos on solid medium containing *cis*-1,4-polyisoprene in the form of latex. The first gordoniae capable of utilizing *cis*-1,4-polyisoprene as a source for carbon and energy were described by Linos and Steinbüchel (63). Surprisingly, these isolates did not produce clear zones on latex-containing solid medium but nevertheless degraded the substrate more effectively. Presumably, this group of rubber-degrading bacteria was not recognized earlier due to the method used for enrichment and characterization. Later studies resulted in the description of the novel species *Gordonia polyisoprenivorans* (62) and *G. westfalica* (64), which are characterized by adhesive growth during rubber degradation. The molecular basis of rubber degradation is still unknown, but one can presume that the hydrophobicity of the cell surfaces of gordoniae affected by the presence of mycolic acids is significantly involved in this process. Probably, the occurrence of mycolic acids and the production of biosurfactants (see below) are important for the formation of biofilms enabling direct contact with *cis*-1,4-polyisoprene in solid rubber materials, which is required for rubber degradation by these strains.

ANABOLIC CAPABILITIES

Gordonia species also produce a variety of compounds which are useful for various applications. The number of publications describing novel compounds produced by *Gordonia* strains has increased steadily. These compounds include L-lysine and some of its analogues (56) and also complex molecules such as gordonan. Some of these are synthesized exclusively by *Gor-*

donia strains; in other cases, *Gordonia* strains may represent an alternative source.

Biosurfactants. The capability for biodegradation of water-insoluble and hydrophobic compounds is often associated with the production of biogenic surface-active, amphiphilic compounds (biosurfactants) by a bacterium (6). The relevance of biosurfactants to the biodegradation of pollutants is threefold: (i) cellular biosurfactants such as mycolic acids cause adherence of the microbial cells to hydrophobic phases in two-phase systems (73); (ii) surfactants promote the access of hydrophobic compounds to microbial cells by decreasing the interfacial tension between the phases (29); and (iii) surfactants disperse hydrophobic compounds, leading to an increased surface area accessible for microbial attack (30). On the other hand, biosurfactants may cause undesired mobilization and movement of hydrophobic pollutants in natural environments, resulting in, e.g., the release of the pollutants into groundwater.

In general, biosurfactants can be subdivided into low-molecular-weight compounds such as glycolipids and lipopeptides and high-molecular-weight polymeric compounds such as polysaccharides, lipoproteins, and lipopolysaccharides. Production of surface-active compounds has been reported for several *Gordonia* strains. In this regard *G. amarae* is the most extensively studied species of the genus. By morphological and physiological means, it has been shown that *G. amarae* is strongly associated with foaming activated sludge in wastewater treatment plants, as also confirmed by phylogenetic hybridization-based experiments and comparative rRNA sequence analysis (20, 21). Pagilla et al. (75) confirmed that foaming problems in activated sludge are due to the production of biosurfactants by *G. amarae* when hydrophobic substrates such as hexadecane are present. They found that the lowest surface tension was 55 dynes/cm in the presence of acetate and less than 40 dynes/cm when hexadecane was provided as the sole source of carbon. It was also demonstrated that foam formation depended on the presence of both bacterial cells and the culture supernatant (39). The authors identified a high-molecular-weight fraction ($M_w > 10,000$) of the supernatant that was able to emulsify *n*-hexadecane in addition to exhibiting foaming activity, indicating the presence of surface-active compounds (39). They also suggested that the hydrophobicity of the cell surface was involved in the attachment of cells to air bubbles, generating stable foam due to the high affinity of the microbial cells for hexadecane. Nazina et al. (72) characterized the formation of biosurfactants by *Gordonia* sp. strain 321, along with that by several strains of different genera. Culture broth of cells grown on a mixture of liquid paraffins significantly decreased the surface tension and interfacial tension, while increasing emulsifying activity, in two-phase systems consisting of an aqueous phase (culture broth) and oil. *Gordonia* sp. strain 321 produced exopolysaccharides, which are known to exhibit surface activity and may be responsible for the results obtained. Another exopolysaccharide-producing *Gordonia* strain, isolated from polluted subtropical groundwater, was identified as *G. polyisoprenivorans* (31). These examples demonstrate that biosynthesis of biosurfactants is important for microbial cells and influences access to hydrophobic substrates.

Carotenoids. The abilities of several gram-positive bacteria, including species of the genera *Corynebacterium*, *Flavobacterium*, *Micrococcus*, and *Mycobacterium*, to synthesize different



FIG. 3. Tn5096-induced mutants of *G. polyisoprenivorans* DSM 44266 with modified carotenoid biosynthesis. Mutants 44-07 and 49-05 were defective in carotenoid synthesis; mutants 33-45 and 38-33 exhibited a coloration slightly different from that of the wild type; and mutant 47-46 obviously overproduced a red carotenoid.

carotenoids are known (19, 40, 54, 77). Many *Gordonia* species also produce colonies with reddish pigmentation, indicating their capacity to synthesize significant amounts of carotenoids (Fig. 3; see also below). *Gordonia jacobaea* MV-1, which is not yet validly described, was isolated because of its property of producing large amounts of carotenoids (22) and was the first *Gordonia* strain characterized with regard to carotenoid biosynthesis. *G. jacobaea* MV-1 was found to contain approximately 200 μg of the ketocarotenoid all-*trans*-canthaxanthin (4,4'-diketo- β -carotene)/g (dry weight) and smaller amounts of all-*trans*-astaxanthin (3,3'-dihydroxy-4,4'-diketo- β -carotene) as a product of later steps in carotenoid synthesis (4). Both compounds are currently produced by Hoffmann-La Roche, Ltd., and are approved by the Food and Drug Administration as food additives in poultry and fish feeding. Carotenoid-overproducing strains were obtained after ethyl methanesulfonate treatment, yielding hyperpigmented mutants with a carotenoid content 10-fold-higher than that of the wild type (23). Because canthaxanthin, unlike other carotenoids, is not converted to vitamin A, and because it leads to crystalline deposition at the retina, its content in animal feed has been limited to 25 mg/kg of body weight by the European Union. In contrast, astaxanthin is still categorized as harmless. Engineering of carotenoid-overproducing strains of *Gordonia* species could provide a new biological source for these economically important substances.

Imidazol-2-yl amino acids. Substituted imidazoles exhibit manifold biological activities and have been extensively used as pharmaceuticals. They can act as antihistamine drugs, defined as H₁, H₂, and H₃ receptor antagonists with structures related to histamine (10, 83), and they are often used as pharmaceuticals against allergic diseases and insomnia because of their distinctive sedating properties. Some substituted imidazoles

exhibit antifungal properties or act as anticonvulsants. Among 61 alkane-oxidizing bacterial strains tested for their abilities to oxidize *N*-(2-hexylamino-4-phenylimidazol-1-yl)-acetamide to imidazol-2-yl amino acids applicable for pharmaceutical purposes, 5 strains producing high yields of the desired products were identified (68). Two of these strains belonged to the genus *Gordonia* and were taxonomically characterized as *G. rubropertincta* and *G. terrae*, respectively. Each strain was able to transform *N*-(2-hexylamino-4-phenylimidazol-1-yl)-acetamide to 1-amino-4-phenylimidazol-2-yl-4-aminobutanoic acid. Two different pathways comprising several enzymatic steps were revealed.

Gordonan, an acidic, cell aggregation-inducing polysaccharide. In general, many bacterial species are able to produce extracellular polysaccharides in response to specific environmental circumstances. Well-known examples are *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*, producing acidic polysaccharides including alginate as a main component to form sessile communities such as biofilms (53). The formation of these biofilms is considered essential for the pathogenicity of these bacteria. During screening for bioactive metabolites produced by microorganisms affecting the BM-N4 insect cell line in a study of insect cell growth and hormone signaling, *Gordonia* sp. strain Y-102, synthesizing a novel acidic polysaccharide referred to as gordonan, was isolated (53). BM-N4 is an insect cell line established from an ovarian tissue of the domestic silkworm *Bombyx mori*, and characteristic morphological changes of this cell line are induced by some insect hormones such as ecdysteroids and bombyxin-II. Gordonan, with an average molecular weight of 5×10^6 , is composed of the trisaccharide repeating unit $\rightarrow 3$ -4-*O*-(1-carboxyethyl)- β -D-Manp-(1 \rightarrow 4)- β -D-GlcAp-(1 \rightarrow 4)- β -D-Glcp-(1 \rightarrow). This polymer induced aggregation of BM-N4 cells at concentrations higher than 4 $\mu\text{g}/\text{ml}$. The aggregated cells were alive and proliferated to become confluent upon further incubation. Control experiments with dextran sulfate and hyaluronic acid indicated that the bioactivity of gordonan was not due to its polyanionic structure. Since the genus *Gordonia* is closely related to the genera *Nocardia* and *Mycobacterium*, members of which cause tuberculosis or leprosy (5), and since members of the genus *Gordonia* are also known to be involved in bronchopulmonary disorders (35) or metastatic melanoma (81), gordonan might support the process of infection or the fixation of *Gordonia* spp. to a host, as do biofilms produced by *P. aeruginosa* (53). Whether gordonan also has effects on mammalian cells is still unclear. However, the isolation of *Gordonia* sp. strain Y-102, producing the novel polysaccharide gordonan, demonstrates that gordoniae are potential sources for very specific compounds. Further highly bioactive substances, which could be of great interest to the pharmaceutical industry, may well be detected in *Gordonia* strains in the future.

Gordonin and other glycosylated peptidolipids. During an investigation of cell wall components responsible for the difference between the rough and smooth colony morphologies of *G. hydrophobica* strains isolated from biofilters for waste gas treatment (9), compounds were identified in the fraction of the most-polar lipids of the smooth variant that were lacking in the rough variant, whereas the less-polar lipids were found to be identical (69). The differing cell wall components were identi-

fied as (mono-) glycosylated peptidolipids consisting of a tridecapeptide interlinked by a β -hydroxylated fatty acid to form a cyclic lactone ring structure. The main fraction of glycosylated peptidolipids, for which the name gordonin was proposed, was identified as 3-hydroxyeicosanoyl-L-seryl-L-phenylalanyl-L-seryl-L-seryl-D-alanyl-L-(*O*- β -D-glucopyranosyl)-threonyl-glycyl-D-leucyl-L-valyl-L-seryl-L-phenylalanyl-glycyl-L-valyl lactone (69). Gordonin differed from the other glycosylated peptidolipids in the nature of the β -hydroxylated fatty acid [20:0 (3-OH) versus 22:1 (3-OH)] as well as in one amino acid (D-leucine versus D-phenylalanine). The amphiphilic character of many peptidolipids gives rise to several biological functions of these molecules such as hemolytic properties, antibiotic activities, or enzyme-inhibiting properties. The amphiphilic nature of gordonin is due to the fact that 20:0 (3-OH) fatty acid and several hydrophobic amino acids are clustered at one side of the proposed structure, while several L-serine residues and glucose are located on the opposite side. Therefore, the authors suggested that gordonin is able to confer a hydrophilic cell surface by intercalating with the hydrophobic tail into the very hydrophobic mycolic acid layer, exposing the hydrophilic head of the molecule to the exterior of the cell. As indicated by the different colony morphologies of rough and smooth variants, gordonin and the other peptidolipids may contribute significantly to the physicochemical character of the cell surface and may enable smooth variants of *G. hydrophobica* to grow in suspension in liquid culture. The authors also reported that gordonin could act as a surfactant, dissolving hydrophobic substrates, if it was released to the environment during growth. However, gordonin was not shown to reduce the interfacial tension between air and water or between water and organic solvents.

GENETIC MANIPULATION OF *GORDONIA* STRAINS

Investigation of the interesting metabolic pathways existing in gordoniae and of the genes encoding the respective enzymes was hampered until recently by the unavailability of suitable genetic tools. The first vectors appropriate for gene transfer to *Gordonia* species have been described only recently, by Arenskötter et al. (3); these are based on an origin of replication (*oriV*) of a native *Rhodococcus* plasmid, and genes can be transferred either by electrotransformation or by conjugational transfer. Also recently, Bröker et al. (12) discovered and sequenced a native 101-kbp megaplasmid, pKB1, occurring in *G. westfalica* DSM 44215^T. Evidence was obtained that pKB1 encodes genes essential for cadmium resistance and rubber biodegradation. Based on the *oriV* region of this megaplasmid, *E. coli*-*Gordonia* shuttle vectors suitable for gene cloning and expression in several *Gordonia* species and members of related taxa were constructed (12). However, vectors appropriate for transposon mutagenesis or for generation of *Gordonia* knockout mutants are still lacking. Unpublished studies document that the temperature-sensitive mycobacterial transposon vector pCG79 (36) also transposed in *G. polyisoprenivorans* DSM 44266, but the mutants obtained were unstable and frequently reverted to a wild-type phenotype (M. Arenskötter et al., unpublished data). Suicide transposon vectors harboring Tn5096 (84) look promising for stable transposon-induced mutagenesis. Auxotrophic mutants with defective amino acid-synthesis-

ing pathways and mutants in which carotenoid biosynthesis is affected (Fig. 3) have been isolated by using these vectors and were subsequently characterized at the molecular level (Arenskötter et al., unpublished).

CONCLUDING REMARKS

As clearly indicated by the increasing number and range of publications, patents, and gene sequence depositions relating to the genus *Gordonia* in recent years, this genus is of significant interest in a wide range of fields. The polyphasic approach for taxonomic classification of gordoniae and closely related genera such as *Rhodococcus*, *Mycobacterium*, and *Nocardia* have established the genus *Gordonia* as a relatively stable entity within the mycolic-acid-containing actinomycetes. However, one may expect that additional new species will be isolated and assigned to the genus *Gordonia* and also that several species already isolated are probably mislabeled and will be reclassified as members of this genus in the future.

The biotechnological interest in members of the genus *Gordonia* is mainly due to the diverse range of enzymatic reactions catalyzed by these bacteria that occur seldom or not at all in other organisms. Many potential applications, such as bioremediation, biodegradation, biotransformation, and production of biosurfactants, seem to be associated with the presence of hydrophobic, mycolic-acid-containing cell envelope structures on gordoniae, but so also do pathogenicity and problems such as foam formation in activated sludge. The potential of microbial processes for the bioremediation and biodegradation of toxic and/or xenobiotic compounds is still high, and despite the high costs and economic infeasibility of many processes, there is continuing industrial interest, as indicated by the increasing number of patents relating to the genus *Gordonia*. Beyond doubt, the metabolic capabilities of *Gordonia* species will continue to attract the interest of the chemical biotechnological industry, and novel applications will probably be identified and explored. One must, however, consider that some members of the genus *Gordonia* are opportunistic pathogens, which will restrict the use of these bacteria.

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