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Gordonia: isolation and identification in clinical samples and role in biotechnology

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Abstract *Gordonia* spp. are members of the actinomycete family, and the environment, especially soil, is the natural habitat of this genus of bacteria. *Gordonia* spp. are important for two aspects: first, some *Gordonia* species cause a broad spectrum of diseases in healthy and immunocompromised individuals; second, these bacteria are capable of producing useful secondary metabolites, which may be used in various industries; therefore, discrimination of the genus *Gordonia* from other genera in the actinomycete family is important. Phenotypic and molecular techniques are necessary for accurate identification of *Gordonia* at the species level.

Introduction

General characteristics and relationships with other aerobic actinomycetes

The genus *Gordonia* is a gram-positive, aerobic, catalase-positive, partially acid-fast, and non-motile bacterium (Vidal et al. 2014). This genus of bacteria belongs to the class *Actinobacteria*, order *Actinomycetales*, suborder *Corynebacterineae*, and the family *Gordoniacea*. In 1997, the genera *Corynebacterium*, *Dietzia*, *Rhodococcus*, *Gordonia*, *Williamsia*, *Millisia*, *Mycobacterium*, *Nocardia*, *Skermania*, *Smaragdicoccus*, *Tsukamurella*, and *Segniliparus* were admitted to the suborder *Corynebacterineae* (Arenskötter et al. 2004; Kageyama et al. 2006; Drzyzga 2012). The results of phenotypic tests in *Gordonia* spp. include the following: arylsulfatase-negative, weakly acid-fast bacilli, non-motile, short rods or circular, sensitive to lysozyme, and G + C ratio from 63–69%. Some *Gordonia* species produce pigmented colonies with colors of orange to orange-red and shiny surfaces (Kummer et al. 1999; Goodfellow et al. 2012). *Gordonia sputi* colonies are smooth, mucoid, and sticky on the medium, whereas *Gordonia bronchialis* colonies are large and dry. Some strains, such as *Gordonia alkanivorans* DSM 44369T and *Gordonia westfalica* DSM 44215T, produce both types of smooth and rough colonies (Arenskötter et al. 2004). In the genus *Gordonia*, the urease test is positive and does not produce aerial hyphae, except for *Gordonia amarae* and *Gordonia defluvii* (Lesens et al. 2000; Shen et al. 2006; Soddell et al. 2006; Goodfellow et al. 2012).

History

In 1971, Tsukamura et al. identified using biochemical tests the genus *Gordona* as a new genus of bacteria that was isolated from sputum or soil. Initially, this organism was placed in the genus *Rhodococcus* due to the similarities in biochemical test results obtained. The genus *Gordona* was again identified by Stackebrandt et al. in 1988 by molecular analysis of the 16S ribosomal RNA (rRNA) gene. Stackebrandt et al. renamed *Gordona* to *Gordonia* to honor an American microbiologist called Ruth E. Gordon in 1997 (Stackebrandt et al. 1988; Stackebrandt et al. 1997; Arenskötter et al. 2004; Franzetti 2007). Recently, 38 species have been identified in the genus *Gordonia* (http://www.bacterio.net/gordonia.html).

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Isolation and identification of *Gordonia* spp.

Isolation methods

The use of enrichment media is an efficient technique for the isolation of the genus *Gordonia*. Enriched medium is the specific medium designed to grow fastidious organisms, which have unusual requirements for growth. For example, an enrichment culture using varying high-salt concentrations provides conditions for the isolation of *Gordonia*. The halophilic species thrive on the salty media, while other organisms do not have the ability to grow. *Gordonia* strains are isolated by this culture. *Gordonia* use different complex carbon sources, such as hexadecane, which provide a starting point and selective media for the isolation of *Gordonia*. *Gordonia* species can grow in complex medium up to 6% (*w*/*v*) NaCl and under aerobic conditions at 28 °C. This bacterium is able to grow at 13–40 °C. Investigations have shown that the optimal temperature for the growth of *Gordonia* species is 28 °C and the optimal NaCl concentration is 2–4% (*w*/*v*) (see Table 1) (Kummer et al. 1999).

Identification (phenotypic and molecular methods)

Phenotypic method (morphology and biochemical characterizations) The phenotypic and biochemical tests for the discrimination of the genus *Gordonia* from other aerobic actinomycetes include the following: partially acid-fast staining, evaluation of aerial hyphae and the sensitivity or resistance to lysozyme, urea hydrolysis, and the production of nitrate reductase (Kummer et al. 1999). The growth of *Gordonia* on different concentrations of sodium chloride, at different temperatures, and the use of carbohydrates and amino acids are one of the best methods for the isolation and identification of the genus *Gordonia* (Kummer et al. 1999; Iida et al. 2005; Kageyama et al. 2006; Goodfellow et al. 2012). The identification of aerobic actinomycetes, such as *Gordonia*, *Rhodococcus*, and *Nocardia*, from clinical samples has remained a significant challenge leading to misclassification (Lai et al. 2010; Drzyzga 2012). There are some features of *Gordonia* that cause difficulties in identifying them to the genus and species level. The second factor, the similarity of phenotypic test results between *Gordonia* and other medically important gram-positives, such as *Rhodococcus* or *Nocardia* and sometimes *Corynebacterium*, can be a cause of misidentification; therefore, the phenotypic tests are of low accuracy and often unreliable. Moreover, the slow growth of these bacteria may inhibit the effect of antimicrobial agents and they can also be lost from clinical samples due to the incubation time of less than 72 h in the medical laboratory (Sng et al. 2004; Werno et al. 2005; Blanc et al. 2007; Brust et al. 2009).

Molecular methods Various molecular methods, such as 16S rRNA gene sequencing (8FLP, 5′-AGAGTTTGATCCTGGCTCAG-3′), (1492RPL, 5′-GGTTACCTTGTTACGACTT-3′), PCR-RFLP of the *hsp65* gene (TB11, 5′-ACCAACGATGGTGTGTCCAT-3′) and (TB12, 5′-CTTGTCGAACCGCATACCCT-3′), use of restriction enzymes (such as *Msp*I and *Hin*fI) (Lai et al. 2010), *gyrB* gene sequencing (F, 5′-ATGGCCTTCCTCAACAAGGG-3′ and R, 5′-GTTCCACTGCATCGCGATCT-3′), and DNA-DNA hybridization, have been used for accurate identification to the species level specially for novel species identification (Steingrube et al. 1997; Sng et al. 2004; Kageyama et al. 2006; Jannat-Khah et al. 2009; Lai et al. 2010). Almost all *Gordonia* species can be identified by 16S rRNA gene sequence determination. Carbon chain lengths of the mycolic acid and menaquinone type are other methods for the discrimination of *Gordonia* from other similar bacteria such as *Corynebacterium*, *Rhodococcus*, and *Tsukamurella* (Linos et al. 1999). A reference laboratory is required for the species-level identification of clinical isolates because not all laboratories have the equipment and skillful specialists to perform these procedures (Brust et al. 2009).

Clinical features of *Gordonia* spp.

Gordonia spp. are responsible for a wide range of diseases in healthy and immune disorder-diseased individuals (Lai et al. 2010). This bacterium is a soil saprophyte isolated from the intestinal contents of mammals (Gil-Sande et al. 2006). Members of the genus *Gordonia* are transferred through aerosols from environmental sources to the respiratory tract. Therefore, the lungs are the primary site of infection. Immunocompromised and immunosuppressive diseases, such as farmer's lung disease, Hodgkin's disease, and surgery associated with coronary artery disease, are risk factors for *Gordonia* infections, and the disease can spread to other organs, including the central nervous system, cardiac infection, and inflammation of the outer ear, otitis, arthritis, and bacteremia. *G. sputi*, *G. bronchialis*, and *Gordonia terrae* are major pathogens in the *Gordonia* species (Herath et al. 2000). *G. sputi* and *G. terrae* were isolated from blood, soft tissue, ocular keratitis and keratoconjunctivitis, brain abscesses (Drancourt et al. 1994, 1997), granulomatous mastitis (Zardawi et al. 2004), metatarsal osteomyelitis disease (Nawaz et al. 2010), and skin infections with lymphadenitis (Lesens et al. 2000). *G. sputi* plays an important role in primary cutaneous and systemic infections in healthy individuals and disorders of the immune system, respectively. Clinical signs of gordonial systemic infections are similar to those of nocardiosis (Blanc et al. 2007). Kuwabara et al. reported *G. sputi* as a cause of mediastinitis after coronary heart bypass surgery in 1999. Richet et al. reported sternal wound infection with *G. bronchialis* in 1991 (Kuwabara et al. 1999). Isolation

Table 1 Biochemical and phenotypic characteristics of the *Gordonia* species (http://www.bacterio.net/gordonia.html; httpt://www.dsmz.de; Brandão et al. 2001; le Roes et al. 2008; Kämpfer et al. 2011; Liu et al. 2011; Kämpfer et al. 2013; Cha and Cha 2013; Luo et al. 2007; Yassin et al. 2007; Shen et al. 2006; Srinivasan et al. 2012; Park et al. 2009; Kang et al. 2014; Yoon et al. 2000; Li et al. 2014; de Menezes et al. 2016; Hamid et al. 2015; Linos et al. 1999)

Species	Optimum temperature (°C)	NaCl concentration (%)	Carbon utilization								Enzymes										
			Glu	Ara	Xyl	Ino	Man	Rha	Raf	Cel	Nit	Pyr	Pal	α-Glu	Ure	Gel	Cit	Arg	Lys	Odc	Vp
G. bronchialis		2.5	+	−	−	+	−	−	−	−	+	+	+	+	+	−	+	+	+	−	+
G. terrae	37	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
G. sputi	37	2.5	+	+	+	+	+	+	+	+					+	−	+	−	−	−	−
G. aichiensis	37	0	+	−	−	−	+	−	−	−					+	−	−	−	−	−	+
G. hydrophobica		7.5	−		−		−				+	−	−	−	−	−					
G. hirsuta		5	+	+	+	+	+	+	+	+					−	−	+	−	−	−	+
G. wesfalica	28		−	−	−	−	+	−	−	+	−	−	+	+	+	−					
G. alkanivorans	28	5	+	−	−	−	+	−	−	+	+	−	+	+	+	−	+	+	+	+	−
G. rhizosphera	28		+	−	−	+	+	+	−	−	−	−	−	−	+	−					
G. amicalis	28		+	−	−	−	+	−	−	−	−	+	+	+	−	−					
G. desulfuricans	30		+	+	−	−	+	+	−	+	+	+	+	+	+	−					
G. sihwensis	28		+	−	−	−	+	−	−	−	+	+	+	+	−	−					
G. paraffinivorans	28		+	−	−	−	−	−	−	−	−	+	+	+	+	−					
G. otitidis	28		+	−	−	−	+	+	−	−	+	−	−	+	−	−					
G. effusa	28		−		−		−				−	+	+	+	−	−					
G. araii	28		−		−		−				−	−	+	+	−	−					
G. defluvii	28		−		−		−				+	−	+	+	−	−					
G. soli	28		−		−		−				−	−	+	+	+	−					
G. kroppenstedtii	28		+	+	+	+	+	+	−	−	−	−	+	+	−	−					
G. cholesterolivorans	28		−		−		−				+	+	+	+	−	−					
G. alkaliphila		0	+		−		+		−	−	+			+		−	−				
G. amarae	20	0	+	−		+[w]	−							+			−				
G. caeni	20	10	+	+		+	+				+			+	−	+[w]	−				
G. didemni	28	1–7		+	+	+	+	+	+					+		+					
G. hankookensis	30			+				+			+				−	−				−	
G. humi			+			−		−									+				
G. iterans	30	0–9	−		−						+	+		+	−	+					
G. jinhuaensis	28–32	0–9	+			−	+	−			+			+	+	−	−				
G. lacunae			+			+[w]	+		+[w]	+	+				+[w]	−					
G. malaquae	22–37		+	−	+	−		−	−	−					+	−	+				
G. namibiensis			+	−	+	+	+			−	−				+	+					
G. neofelifaecis	30							+									−				

Table 1 (continued)

Species	Optimum temperature (°C)	NaCl concentration (%)	Carbon utilization							Enzymes							
			Glu	Ara	Xyl	Ino	Man	Rha	Raf	Cel	Nit	Pyr	Pal	α-Glu	Ure	Gel	Cit	Arg	Lys	Odc	Vp
G. nitida	30–37		+			–		–			–				+		+				
G. phosphorivorans			$+^{w}$	–	–	–	–	–		–							–				
G. polyisoprenivorans	28–35					+		+									+				
G. rubripertincta			–		–		+				–			+	+						
G. shandongensis	20–37			+				–		+	+						–				
G. sinesedis	37									–	–				±						

+: positive, $^{-}$: negative, w: weak

Glu glucose, *Ara* arabinose, *Xyl* xylose, *Ino* inositol, *Man* mannose, *Rha* rhamnose, *Raf* raffinose, *Cel* cellobiose, *Nit* nitrate, *Pyr* pyrrolidonyl arylamidase, *Pal* phenylalanine ammonia lyase, *α-Glu* alpha-glucosidase, *Ure* urease, *Gel* gelatinase, *Cit* citratase, *Arg* arginase, *Lys* lysine synthetase, *Odc* ornithine decarboxylase, *Vp* Voges-Proskauer

of this bacterium from sternal wounds is difficult because it is mixed with other microorganisms. As well, *Haemophilus aphrophilus* inhibits the growth of *G. bronchialis* in the laboratory (Richet et al. 1991; Sng et al. 2004; Nguyen et al. 2014). *Gordonia polyisoprenivorans* is a cause of bloodstream infections. Multiple factors, such as the use of a long-term catheter, may provide conditions for bloodstream infections created by *G. polyisoprenivorans* in immunocompromised patients (Kempf et al. 2004; Verma et al. 2006; Gupta et al. 2010; Moser et al. 2012). Additionally, infections occur such as endocarditis, pleural infection, and abscesses in the paravertebral and breast by *G. sputi*, *G. polyisoprenivorans*, *G. bronchialis*, and *G. bronchialis*, respectively (Lesens et al. 2000; Sng et al. 2004; Werno et al. 2005; Verma et al. 2006; Vidal 2014). Cutaneous infection by *Gordonia amicalis* was reported in trauma and injury (Lai et al. 2012). In 2009, the first case of *Gordonia araii* reported in healthy individuals was isolated from a medical device (Jannat-Khah et al. 2009). The association between *Gordonia* species and clinical diseases is shown in Table 2.

Pathogenesis and virulence factors

The genus *Gordonia* is an opportunistic pathogen and is able to produce certain secondary metabolites and can also consume complex carbon sources. Some species of *Gordonia* can be attached and colonized to the appropriate surface by biofilm formation. Immunocompromised patients cannot defend themselves against this infectious agent, and it may be the leading cause of bacteremia. For example, *G. polyisoprenivorans* produces biosurfactants and these compounds help to form biofilm onto the catheter (Kempf et al. 2004; Verma et al. 2006; Gupta et al. 2010; Moser et al. 2012). Biofilm causes decreased penetration of various antibiotic agents, which leads to antibiotic resistance (Werno et al. 2005).

Cell wall structure of *Gordonia*

The cell wall in *Gordonia* spp. is composed of a chemotype IV structure. Ingredients in the cell wall of the genus *Gordonia* include meso-diaminopimelic acid, arabinose, galactose, and N-glycosylated muramic acid, which are part of peptidoglycan (Takeuchi and Hatano 1998). The carbon chain in mycolic acid has 40–66 carbons (Nishiuchi et al. 2000). Types of fatty acids in cell wall structures include tuberculostearic acid, saturated fatty acids, and unsaturated fatty acids (Linos et al. 1999).

Association with infections in human

Susceptibility and therapeutic approaches

Previous studies have shown that 89% of pathogenic *Gordonia* species are sensitive to vancomycin (Ma et al.

2014). In 2012, the antibiotic susceptibility profile of this microorganism showed that 57% were resistant to trimethoprim-sulfamethoxazole (Moser et al. 2012). Gordonial infections may be misdiagnosed as nocardiosis due to the low prevalence of *Gordonia* infections and similar clinical manifestations, which is significant and may lead to treatment failure. Methods that have been used for the determination of the antimicrobial susceptibility testing of pathogenic *Gordonia* species include the broth microdilution method, E-test (Pham et al. 2003; Kofteridis et al. 2011), and disk diffusion method (Lesens et al. 2000). The antibiotic susceptibility of *Gordonia* is similar to that of *Rhodococcus*. However, although vancomycin is used for the treatment of *Rhodococcus* infections, previous studies have shown that 11% of *Gordonia* isolates were resistant to vancomycin. Therefore, antibiotic susceptibility testing for any isolate is necessary and important (Renvoise et al. 2009). Catheter removal is recommended for the treatment of gordonial infections in children (Ma et al. 2014). There are no standardized suggestions for the treatment of these infectious diseases. The literature has shown that *Gordonia* spp., unlike some other actinomycetes, such as *Rhodococcus* spp., are usually susceptible to numerous antimicrobial drugs (Johnson et al. 2011). Previous studies have suggested the combination of penicillin and an aminoglycoside as the appropriate treatment for *Gordonia* bacteremia. Carbapenem or fluoroquinolones can also be used in combination with an aminoglycoside (Pham et al. 2003; Iida et al. 2005; Gil-Sande et al. 2006; Blaschke et al. 2007; Renvoise et al. 2009; Johnson et al. 2011; Ma et al. 2014). Antibiotic resistance to common antibiotics for the treatment of opportunistic bacteria, such as *G. polyisoprenivorans*, has been reported (Kempf et al. 2004; Verma et al. 2006; Gupta et al. 2010; Moser et al. 2012). Aoyama et al. reported trimethoprim-sulfamethoxazole and some of aminoglycoside agents were effective on some of *Gordonia* species and they were resistant to fosfomycin (Aoyama et al. 2009).

Biotechnological/commercial potential catabolic products

Catabolism is the spontaneous process by which organisms utilize complex carbon sources and generate energy. Some

Table 2 Association between *Gordonia* species isolated from human and clinical diseases (Richet et al. 1991; Pham et al. 2003; Kempf et al. 2004; Sng et al. 2004; Iida et al. 2005; Werno et al. 2005; Gil-Sande et al. 2006; Kageyama et al. 2006; Verma et al. 2006; Aoyama et al. 2009; Jannat-Khah et al. 2009; Renvoise et al. 2009; Nawaz et al. 2010; Johnson et al. 2011; Kofteridis et al. 2011; Lai et al. 2012; Ramanan et al. 2013)

Species	Commonly associated disease				Underlying disease
	Primary skin	Pulmonary	Presence of bacteria in the blood	Reference (s)	
G. sputi	+		+	Kofteridis et al. (2011)	Breast cancer
			+	Renvoise et al. (2009)	Mediastinitis (after coronary artery bypass grafting (CABG))
G. bronchialis	+		+	Werno et al. (2005)	Breast abscess (immunocompetent)
				Sng et al. (2004)	Sequestrated lung
	+	+	+	Richet et al. (1991)	Sternal wound infection (after CABG)
				Johnson et al. (2011)	Pleural infection
G. terrae	+		+	Gil-Sande et al. (2006)	Acute cholecystitis
			+	Pham et al. (2003)	Leukemia or solid tumor
				Nawaz et al. (2010)	Immunocompetent (metatarsal osteomyelitis)
G. polyisoprenivorans			+	Verma et al. (2006)	Native valve endocarditis
			+	Kempf et al. (2004)	Bone marrow transplantation
G. amicalis	+			Lai et al. (2012)	After cutaneous infection
G. araii		+		Kageyama et al. (2006)	Bacterial pneumonia
				Jannat-Khah et al. (2009)	Orthopedic device
G. effusa		+		Kageyama et al. (2006)	Kidney dysfunction
G. otitidis	+		+	Iida et al. (2005)	External otitis
				Ramanan et al. (2013)	Acute monoblastic leukemia
G. aichiensis		+		Aoyama et al. (2009)	Pulmonary infection

Plus (+) is positive

Gordonia species are capable of decomposing various aromatic hydrocarbons and the use of these compounds as a carbon source for growth. These hydrocarbons include polycyclic aromatic hydrocarbons, the xenobiotics, the alkyl pyridines, the phthalates, and naphthalene. They are very dangerous and carcinogenic compounds that have been achieved with chemical interactions (Drzyzga 2012). These valuable phenotypic features of some *Gordonia* spp. can be transferred to other nonpathogenic microorganisms by genetic transformation, a useful property in the biotechnology industry (Arenskötter et al. 2004).

Anabolic compounds

Anabolism is the process by which organisms utilize the energy produced by catabolism to synthesize complex materials. These bacteria produce various anabolic compounds, including bendigoles, L-lysine, ethyl acetate, biosurfactants, carotenoids, imidazol-2-yl amino acids, and gordonan, which are useful in many applications (Arenskötter et al. 2004).

Bendigoles

Bendigoles are the first secondary metabolites produced by the genus *Gordonia*. They have been identified from the cultures extracted from *Gordonia australis*, which can produce three types of bendigoles (A, B, and C). The A and B types are only able to bind to the receptors of androgenic hormones, and the C type is able to bind to the receptors of progesterone and the androgen hormones. A, B, and C types cannot bind to estrogen or mineralocorticoid receptors (Schneider et al. 2008).

Biosurfactants

Biosurfactants are surface-active compounds that are produced in two forms: extracellular and cell-bound. Biosurfactants have a variety of chemical structures, including glycolipids, fatty acids, phospholipids, lipopeptides, lipoproteins, and the polymeric specific structures. Biosurfactants have strong antimicrobial properties, environmental pollution degradation capabilities, and anti-adhesive activity against pathogenic bacteria, indicating that this role of the bacteria may help to treat bacterial diseases. Biosurfactants are also able to decrease the biofilm produced by pathogenic bacteria such as *Salmonella typhimurium*, *Salmonella enterica*, *Escherichia coli*, and *Proteus mirabilis*. The antimicrobial properties of biosurfactants produced by *Bacillus cereus* have been investigated in other bacteria, but unfortunately, no study has been performed on the biosurfactants generated by *Gordonia* spp. (Singh and Cameotra 2004; Mukherjee et al. 2006).

Pigment production

Gordonia jacobaea grows on blood agar after 3–7 days. It produces colonies with brown, pink, orange, and red colors. Chromatic colonies are created by pigment production in this species of bacterium. *G. jacobaea* can produce carotenoid pigments. Canthaxanthins are a type of pigment that are used in the food and cosmetic industries (Arenskötter et al. 2004; Veiga-Crespo et al. 2010).

Other metabolites

Some *Gordonia* species have the ability to oxidize alkanes to produce these compounds. These compounds act as antihistamines that compete with the histamine receptors (H1, H2, and H3). Imidazol-2-yl amino acids, due to their distinctive properties, may be used in the pharmaceutical industry for the treatment of allergic and insomnia diseases in the future (Mikolasch et al. 2003; Drzyzga 2012). Gordonans are polysaccharide compounds with high specificity that are produced by *Gordonia*. Fortunately, they may be used as indicators to identify *Gordonia* strains in the future. Also, Gordonan can form sessile communities due to induction of the cell aggregation properties of polysaccharides. They are effective in the stabilization and infection process in the host (Kondo et al. 2000). Therefore, Gordonan may be considered as a cause of treatment failure (Werno et al. 2005).

Conclusion

We can conclude that the identification of *Gordonia* is important, because the genus *Gordonia* is the opportunistic pathogen that causes diseases in the lung and skin and, in severe cases, can lead to bacteremia. Cooperation between doctors and microbiologists is essential for the rapid isolation of this genus of bacteria from other similar genera and is recommended for long-term treatment, timely use, and combinations of antibiotics (Ma et al. 2014). The identification of these bacteria is difficult using phenotypic methods (Kondo et al. 2000). Molecular methods are important for the accurate identification of *Gordonia* species for appropriate treatment (Arenskötter et al. 2004). Most *Gordonia* species are capable of decomposing xenobiotics and other environmental pollutants. They can also produce the valuable secondary metabolites mentioned above. In this respect, this genus of bacteria has many applications in the medical, pharmaceutical, environmental, and biotechnological industries, which is encouraging to researchers. Cooperation and teamwork for research projects at universities of medical sciences and basic sciences, such as biotechnology, are needed for a better understanding and to demonstrate the instrumental importance of this genus

of bacteria. We propose to study all aspects and hope to achieve practical success in the future.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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