

Original Research Article

Characterization of *Gordonia sinesedis* isolated from a zebu cow suffering from lymphadenitis

Abstract

Most of the *Gordonia* species described earlier have been considered as opportunistic pathogens in humans, but recently described species have been isolated from the environment with notable roles in bioremediation or the biodegradation of pollutants. A gram-positive slightly acid-fast nocardioform bacterial, strain SD256 (DSM 45847), was isolated from a granulomatous lymph node of a zebu cow at Kaduqli, western Sudan, was subjected to a polyphasic taxonomic study. The strain was found to have morphological, biochemical and chemotaxonomic properties that were consistent with its assignment to the genus *Gordonia*. Although the strain exhibited some phenotypic variations from the type strain of *Gordonia sinesedis*, 16S rRNA gene sequencing (accession: KC895879) and DNA-DNA relatedness showed 100% and 99.6% similarity, respectively. Microbiologic diagnoses of fastidious actinomycetes such as *Gordonia* species are often difficult and challenging. However, the combined phenotypic and genotypic data confirm that this strain is a member of *Gordonia sinesedis*, which represents a first record of gordonia infection in farm animals.

Keywords: *Gordonia sinesedis*, nocardioform, actinomycetes, lymphadenitis, zebu cattle, Sudan

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26 1. INTRODUCTION

27 *Gordonia* is a member of the mycolic acid-containing aerobic actinomycetes,
28 which was first described in 1971 for coryneform bacteria isolated from patients
29 with pulmonary disease or from soil [1]. The genus belongs to suborder
30 *Corynebacterineae* within the order *Actinomycetales* [2] and currently contains
31 30 recognized species including some human pathogens [3, 4]. Most of the
32 *Gordonia* species described earlier have been considered as opportunistic
33 pathogens in humans [3, 4]. But recently described species have been isolated
34 from the environment with notable roles in bioremediation or the biodegradation
35 of pollutants [5, 6, 7].

36 Mycolic acid containing actinomycetes are a group of closely related acid fast to
37 weakly acid fast actinomycetes that belongs to the genera *Corynebacterium*,
38 *Dietzia*, *Gordonia*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Seigniliparus*,
39 *Skermania*, *Tsukamurella* and *Williamsia* [8]. The majority of species of these
40 actinomycetes are saprophytes but few species cause variety of infections in man
41 and animals [9]. Species frequently associated with animals diseases are mainly
42 *Mycobacterium bovis*, the causal agent of bovine tuberculosis, *M. farcinogenes*,
43 the causal agent of bovine farcy; *M. avium* subsp. *paratuberculosis*, the causal
44 agent of John's disease and *Nocardia farcinica* as a cause of mastitis [10].

45 Microbiologic diagnosis of fastidious actinomycetes such as *Gordonia* spp. is often
46 difficult and challenging. Consequently, a significant numbers of misdiagnosis or

47 incorrect identification of clinical isolates may occur. Identification at the genus and
48 species levels is presently obtained by 16S rRNA sequence comparisons [11].

49 The aim of this study was to clarify the taxonomic position of an actinomycete,
50 designated SD256, isolated from a lymph node of a zebu cow at Kaduqli area,
51 Sudan.

52 2. MATERIALS AND METHODS

53 2.1. The case

54 An enlarged caseated lymph node was collected from a cow that had been
55 slaughtered at Kaduqli slaughterhouse, western Sudan. The cow was a local zebu
56 cow belonging to the Baggara breed. Lymph node was collected and
57 immediately transported to the laboratory for bacteriological characterization.
58 The specimen was collected by Dr Musa Tibin Musa during a survey for bovine
59 farcy between 1977 and 1978 [12].2.

60 2.2. Isolation

61 A swab moistened by 5% oxalic acid (a decontamination method) was used to
62 transfer purulent materials from the peripheries of the capsule of the infected
63 lymph node onto two Lowenstein Jensen (LJ) slants. Two smears were made
64 from the purulent materials for examination using Gram's and Ziehl-Neelsen
65 methods.

66 Inoculated LJ slopes were incubated aerobically at 37°C and examined daily
67 during the first three days, then weekly for up to 4 weeks for bacterial growth.
68 Grown cultures simulating *Nocardia farcinica* (which was considered the causal
69 agent of bovine farcy) were lyophilized and stored for further analysis.

70 **2.3. Phenotypic identification**

71 The isolate labelled SD256 (DSM 45847), along with other bovine farcy
72 isolates, was earlier characterized using selected morphological and biochemical
73 tests [13]. Subsequent subcultures were made onto glucose yeast extract malt
74 extract agar (GYM), glucose yeast extract agar (GYEA) and tryptic soya agar
75 (TSA; Oxoid) for identification based on morphological, biochemical and
76 physiological criteria (Table 1) [14].

77 The strain was examined for many phenotypic properties, particularly for
78 features known to be of value in *Gordonia* systematics [15]. Cultural
79 characteristics were examined on a TSA plate that had been incubated for 21
80 days at 30°C. Smears taken from the plate were Gram-stained and examined for
81 micromorphological properties. Additional smears were stained using a
82 modification of the Ziehl-Neelsen method [16]. Standard biochemical and
83 degradation tests were carried out using previously described procedures [14,
84 17].

85 **2.4. Cell wall analysis**

86 The isomeric form of diaminopimelic acid (meso-DAP) was determined by thin-
87 layer chromatography (TLC) of whole-organism hydrolysates following the
88 procedure described by Stanek and Roberts ([18]. Standard procedures were
89 also used for the extraction and analysis of mycolic acids [19], with the
90 appropriate marker strains used as controls. The presence of single-spot co-
91 chromatographs with reference *G. bronchialis* N654 (DSM 43247^T), confirmed
92 the presence of mycolates of *Gordonia* species.

2.5. 16S rRNA gene sequence analysis

The complete 16S rRNA gene sequence was determined by direct sequencing of PCR-amplified 16S rDNA. Genomic DNA extraction was carried out using the MasterPureTM Gram Positive DNA Purification Kit (Epicentre Biotechnologies) according to the manufacturer's instructions. PCR mediated amplification of the 16S rDNA and purification of the PCR product was carried out as described previously [20]. Purified PCR products were sequenced using the CEQTMDTCS-Quick Start Kit (Beckmann Coulter) as directed in the manufacturer's protocol. Sequence reactions were electrophoresed using the CEQTM8000 Genetic Analysis System.

The resulting sequence data was put into the alignment editor ae2 [21], aligned manually and compared with representative 16S rRNA gene sequences of organisms belonging to the *Actinobacteria* [21]. Obtained 16S rDNA nucleotide sequences data were tested on the BLAST electronic system (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to establish a quick phylogenetic position. Following an assignment of the isolate with *Gordonia* spp. in the BLAST system, the sequences were analyzed using PHYDIT for Windows (Version 3.1., J. Chun) and in comparison to all known sequences of *Gordonia* spp. obtained from GenBank database (<http://www.ncbi.nlm.nih.gov/nucleotides>).

2.6. DNA-DNA hybridization

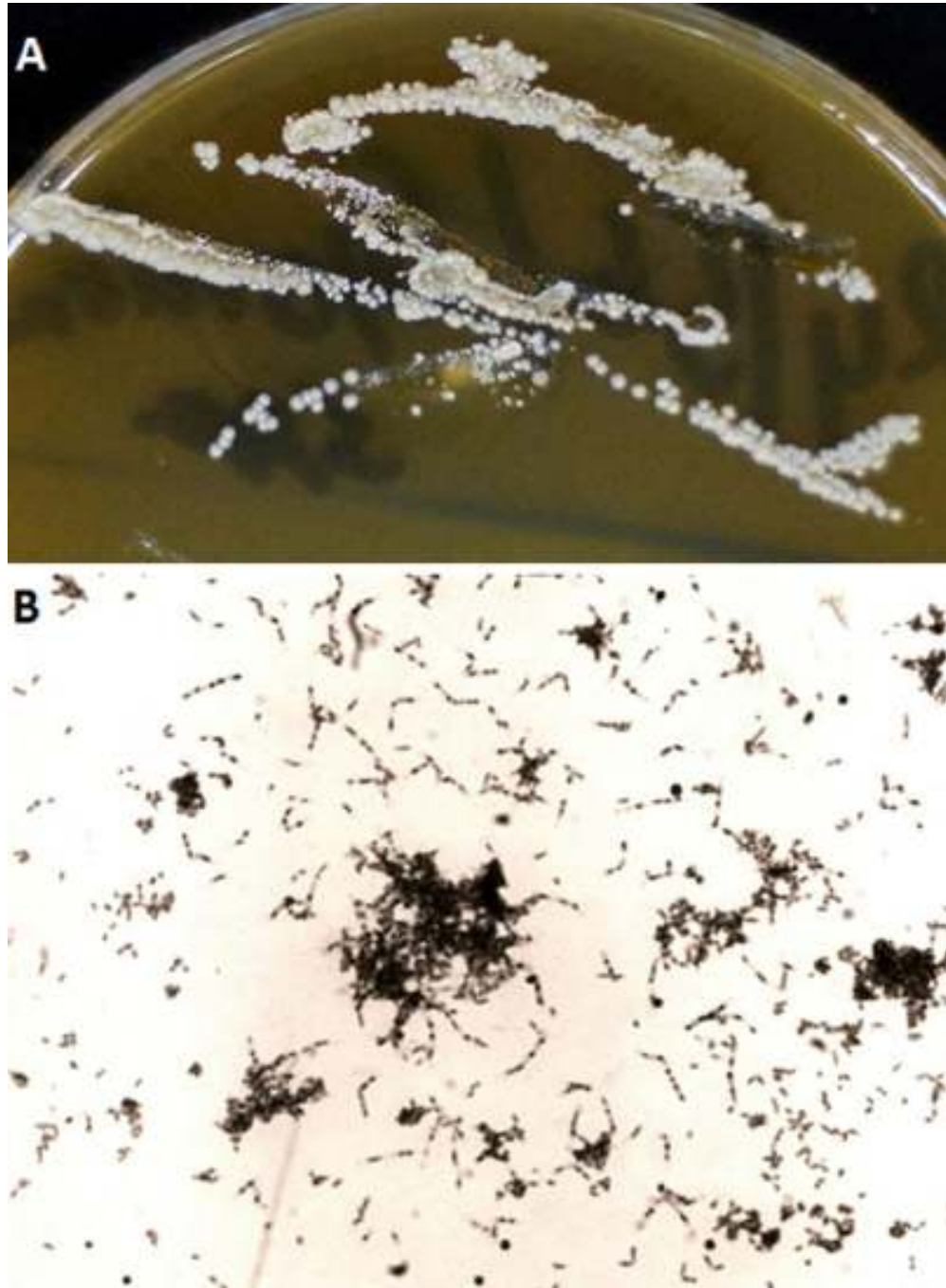
DNA-DNA hybridization *Gordonia* sp. strain SD256 against *Gordonia sinesedis* DSM 44455^T was carried out as described by De Ley *et al.* [22] under consideration of the modifications described by Huss *et al.* [23] using a model

117 Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted
118 6x6 multicell changer and a temperature controller with *in-situ* temperature
119 probe (Varian). Cells were disrupted by using a Constant Systems TS 0.75 KW
120 (IUL Instruments, Germany) and the DNA in the crude lysate was purified by
121 chromatography on hydroxyapatite as described by Cashion *et al.* [24].

122 3. RESULTS

123 3.1. Isolation and phenotypic characterization

124 The strain SD256 was isolated in Lowenstein Jensen medium but subsequently
125 grew in a variety of agar-based media such as GYM agar, GYEA and TSA.
126 Colonies were visible after 2-3 days when incubated aerobically at 37°C. The
127 colonies appeared as creamy, rough with irregular edges or button shape that is
128 loosely attached to the medium (Fig. 1). The isolate is aerobic, gram-positive,
129 weak acid alcohol fast actinomycete that forms branched substrate mycelia.
130 These hyphae disintegrated into rod/coccus-like elements (Fig. 1). Aged cells
131 tended to form aggregates. Cells were non-motile and non-spore forming.



132

133 Figure 1. Colony morphology of *Gordonia* sp. strain SD256 showing the growth of
 134 creamy, rough colonies with irregular edges (top), and microscopic picture of strain
 135 SD256 showing branching filaments (partially acid alcohol fast) which tends to break into
 136 short chains of coccobacillary elements (bottom) (Oil immersion x1000).

137

138 The results of biochemical and physiological tests are shown in Table 1.

139 Table 1. Phenotypic characteristics separating strain SD256 from representatives
140 of validly described *Gordonia* species

Species																
	SD256	Gordonia sinesedis strain J72	G. aichiensis DSM 43978 ^T	G. alkanivorans DSM 44369 ^T	G. amarae DSM 43392 ^T	G. amicalis DSM 44461 ^T	G. bronchialis DSM 43247 ^T	G. desulfuricans DSM 43247 ^T	G. hirsuta DSM 44140 ^T	G. hydrophobica DSM 44015 ^T	G. nitida DSM 44499 ^T	G. polyisoprenivorans DSM 44302 ^T	G. rhizosphaera IFO 16247 ^T	G. rubropertincta DSM 43197 ^T	G. sputi DSM 43896 ^T	G. terrae DSM 43249 ^T
Character	SD256	Gordonia sinesedis strain J72	G. aichiensis DSM 43978 ^T	G. alkanivorans DSM 44369 ^T	G. amarae DSM 43392 ^T	G. amicalis DSM 44461 ^T	G. bronchialis DSM 43247 ^T	G. desulfuricans DSM 43247 ^T	G. hirsuta DSM 44140 ^T	G. hydrophobica DSM 44015 ^T	G. nitida DSM 44499 ^T	G. polyisoprenivorans DSM 44302 ^T	G. rhizosphaera IFO 16247 ^T	G. rubropertincta DSM 43197 ^T	G. sputi DSM 43896 ^T	G. terrae DSM 43249 ^T
Color of colonies	Beige	Beige	Pink/ orange	Pink/ orange	Tan/ white	Red	Brown	Pink	White/ light	Tan/ white	Pink/ orange	Orange	Pink/ orange	Orange/ red	Pink	Pink/ orange
Biochemical tests																
Aesculin hydrolysis	+	-	-	-	+	-	-	-	-	+	-	-	-	-	+	+
Allantoin hydrolysis	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-
Arbutin hydrolysis	+	-	+	-	+	-	-	-	-	+	-	-	-	+	+	+
Nitrate reduction	-	+	+	+	-	-	+	+	+	+	-	-	-	-	+	+
Urea hydrolysis	+/-	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+
Decomposition of (% w/v)																
Hypoxanthine (0.4)	-	+	-	-	+	-	-	+	-	+	-	-	-	+	+	+
Starch (1)	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
Tributyrin (0.1)	Nd	+	-	+	-	-	-	-	-	-	+	+	-	+	-	-
Tween 80 (1)	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
Tyrosine (0.5)	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-
Uric acid (0.5)	Nd	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+
Xanthine (0.4)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Growth on sole carbon sources (% w/v):																
Arbutin (1)	-	-	-	-	+	+	-	+	+	+	-	-	+	-	-	-
D-Cellobiose (1)	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
Glycerol (1)	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+
N-acetyl-D-glucosamine (0.1)	+	+	-	+	+	-	-	-	+	+	-	+	+	+	-	-
Adipic acid (0.1)	-	+	+	-	-	+	-	+	-	+	+	+	+	-	+	-
Betaine (0.1)	Nd	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
Oxalic acid (0.1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Propan-1-ol (0.1)	+	+	-	-	+	-	-	+	-	+	+	-	+	-	-	+
Sodium fumarate (1)	Nd	+	-	+	-	+	+	-	-	-	+	-	+	+	-	+
Oleic acid (0.1)	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Zinc chloride (0.001)	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+

141
142 Data for species other than strain SD256 were taken from Maldonado *et al.* [25].
143 ND, not determined

3.2. Cell wall analysis

On thin layer chromatography the organism contained mycolic acids that co-chromatographed with mycolates from *Gordonia* species (Fig. 2). The isolate showed biochemical, degradative and a nutritional profile that is comparable to those of MACA actinomycetes but close to those of *G. sinesedis* [25].

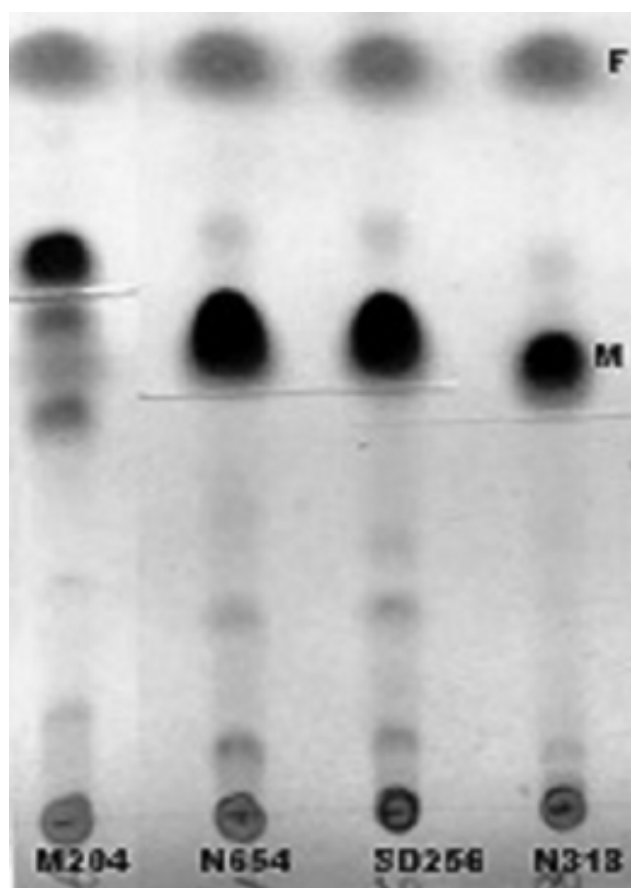
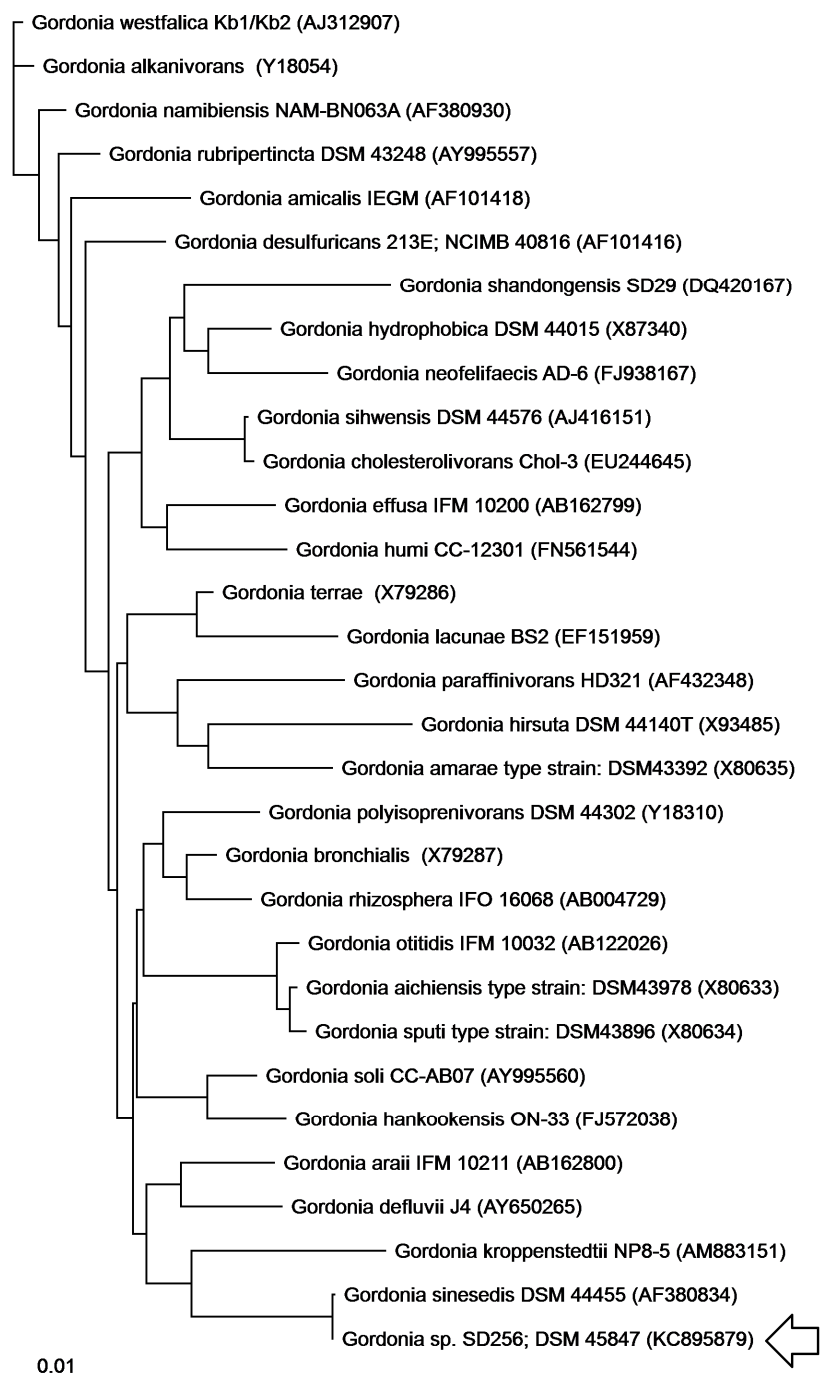


Figure 2. One-dimensional TLC of methyl mycolates of *Gordonia* sp. (SD256) in comparison to *Gordonia bronchialis* (N654), *Nocardia brasiliensis* (N318) and *Mycobacterium fortuitum* (M204). Developing solvent: Petroleum ether (b.p. 60-80 °C)/diethyl ether (85: 15, v/v, x 2). Detection agent, 5% ethanolic molybdophosphoric acid followed by charring at 180 °C. Abbreviations. M, mycolic acid methyl esters; F, non-hydroxylated fatty acid methyl esters.

157 **3.3. 16S rRNA gene sequence analysis**

158 Comparison of the complete 16S rDNA sequences (1517 bp; accession number:
159 KC895879) of the isolate with corresponding nucleotide sequences of
160 representatives of the suborder *Corynebacterineae* showed that the isolate
161 belongs to the genus *Gordonia* (Fig. 3). It shares the highest 16S rRNA gene
162 sequence similarity with *Gordonia bronchialis* DSM 43297^T (97.2), *Gordonia*
163 *polyisoprenivorans* DSM 44302^T (97.1%), *Gordonia araii* IFM 10211^T (97.0%);
164 *Gordonia soli* CC-AB07^T (97.0%) and *Gordonia terrae* DSM 43249^T (97.0%).



165

166 Figure 3. Phylogenetic tree based on sequences derived from 16S rDNA gene showing
 167 the relationship between isolate (SD256; accession, KC895879) and members of the
 168 genus *Gordonia* and related mycolic acid containing actinomycetes.

169 **3.4. DNA-DNA hybridization**

170 DNA-DNA hybridization *Gordonia* sp. strain SD256 against *Gordonia sinesedis* DSM
171 44455^T in 2 X SSC + 10% formamide at 70°C showed a relatedness of 99.6%.

172 **4. Discussion**

173 Members of the genus *Gordonia* are environmental bacteria but their association
174 in human disease is increasing given the recent increase in reports of infections
175 caused by *Gordonia* spp. *Gordonia* spp. are often challenging to identify
176 especially when using few phenotypic criteria. They are often misidentified as
177 *Rhodococcus* spp. or *Corynebacterium* spp. A more accurate method to identify
178 *Gordonia* spp. is considered the application of 16S rRNA gene sequence
179 analysis [11]. This bacterium is often associated with medical devices and could
180 be regarded as a notable nosocomial agent. Thus, it is worthwhile not to consider
181 such isolates as contaminants, and should be thoroughly identified by molecular
182 methods in addition to conventional biochemical tests.

183 The isolate SD256 was found to have cultural and morphological properties simulating
184 those of mycolic acid-containing actinomycetes. Strain SD256 produced short,
185 elementary branching hyphae (Fig. 1) that disintegrated into rod/coccus-like elements,
186 showing the typical rod–coccus growth cycle that is usually found among strains of the
187 genus *Gordonia* and related genera notably *Nocardia*, *Rhodococcus* and *Tsukamurella*
188 [8]. Most of the morphological, physiological and chemotaxonomic characteristics of
189 strain SD256 were coherent with the diagnostic features of the genus *Gordonia* [8, 26]. It
190 is evident from the results in Table 1 that the strain SD256 showed some phenotypic
191 variations from *G. sinesedis*. But, 16S rDNA sequencing and DNA:DNA pairing revealed
192 100% and 99.6% similarity with *G. sinesedis*, respectively. According to the DNA:DNA

193 pairing *Gordonia* sp. strain SD256 belongs to the species *Gordonia sinesedis* DSM
194 44455T. This assignment follows recommendations of a threshold value of 70 % DNA-
195 DNA similarity for the definition of bacterial species by the *ad hoc* committee [27].

196 The isolation of gordoniae from clinical samples has been previously reported in
197 human but, to our knowledge, not from cattle. But, Anderson and Petty [28]
198 isolated *Gordonia* sp. from exudates of seahorses presented with altered
199 buoyancy and distended upper trunks. Maldonado *et al.* [25] described *G.*
200 *sinesedis* as new species, which was isolated from soil. In this report, the strain
201 was isolated from bovine exhibiting typical granulomatous reaction in the
202 affected lymph node. These types of lesions do not differ from those exhibited
203 by *M. farcinogenes* [29, 30].

204 **5. Conclusions**

205 Our data indicate that the strain (SD256) is a member of *Gordonia sinesedis*,
206 which to our knowledge, represents a first evidence of gordonia infection in farm
207 animals. No further clinical, pathological or epidemiological information was
208 available about the case. Thus, further surveys in similar setting must consider
209 rare agents such as the mycolic acid containing actinomycetes, in order to
210 establish their actual prevalence.

211 **Competing interests**

212 The authors declare that they have no financial or personal relationships which
213 may have inappropriately influenced them in writing this article.

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216 **References**

- 217 1. Tsukamura M. Proposal of a new genus, *Gordona*, for slightly acid-fast
218 organisms occurring in sputa of patients with pulmonary disease and in soil. J
219 Gen Microbiol. 1971; 68: 15-26.
- 220 2. Stackebrandt E, Rainey FA, Ward-Rainey NL. Proposal for a new hierarchic
221 classification system, *Actinobacteria* classis nov. Int J Syst Bact. 1997; 47:
222 479–491.
- 223 3. Tsukamura M. Numerical classification of *Rhodococcus* (formerly *Gordona*)
224 organisms recently isolated from sputa of patients; description of
225 *Rhodococcus sputi* Tsukamura sp. nov., Int J Syst Bact. 1978;. 28; 169–181
- 226 4. Blaschke AJ, Bender J, Byington CL, Korgenski K, Daly J, Petti CA, Pavia
227 AT, Ampofo K. *Gordonia* species: emerging pathogens in pediatric patients
228 that are identified by 16S ribosomal RNA gene sequencing. Clin Infect Dis.
229 2007; 45: 483-6.
- 230 5. Arenskötter M, Bröker D, Steinbüchel A. [7]Biology of the metabolically
231 diverse genus *Gordonia*. Appl Environ Microbiol. 2004; 70: 3195-204
- 232 6. Chanthamalee J, Luepromchai E. Isolation and application of *Gordonia* sp.
233 JC11 for removal of boat lubricants. J Gen Appl Microbiol. 2012; 58: 19-31.
- 234 7. Jin D, Bai Z, Chang D, Hoefel D, Jin B, Wang P, Wei D, Zhuang G.
235 Biodegradation of di-n-butyl phthalate by an isolated *Gordonia* sp. strain
236 QH-11: Genetic identification and degradation kinetics. J Hazard Mater.
237 2012; 221-222. 80-85.
- 238 8. Goodfellow M, Maldonado LA. The families *Dietziaceae*, *Gordoniaceae*,
239 *Nocardiaceae* and *Tsukamurellaceae*. In: Dworkin, M., Falkow, S.,
240 Rosenberg, E., Schleifer, K.H. and Stackebrandt, E. (eds). The Prokaryotes
241 3rd Ed., Vol. 3: Archaea and Bacteria: Firmicutes, Actinomycetes. Springer,
242 New York, pp. 843-888. 2006.
- 243 9. Conville PS, Witebsky FG. *Nocardia*, *Rhodococcus*, *Gordonia*,
244 *Actinomadura*, *Streptomyces*, and other aerobic actinomycetes. In Manual of
245 Clinical Microbiology, 9th edn, pp. 515–542. Edited by Murray, P. R., Baron,
246 E. J., Jorgensen, J. H., Landry, M. L. & Pfaller, M. A.. Washington, DC. :
247 American Society for Microbiology. 2007.
- 248 10. Radostits OM, Gay CC, Blood DC, Hincheliff FW. Veterinary Medicine. A
249 text book for the diseases of cattle, sheep, pigs, goats and horses, 10th Ed,
250 Bailliere Tindall, London. 2007.
- 251 11. Wang T, Kong F, Chen S, Xiao M, Sorrell T, Wang X, Wang S, Sintchenko
252 V. Improved identification of *Gordonia*, *Rhodococcus* and *Tsukamurella*
253 species by 5'-end 16S rRNA gene sequencing. Pathology. 2011; 43: 58-63.
- 254 12. El Sanousi SM, Salih MA, Mousa MT, Tag El Din MH, Ali SA. Further
255 studies on the properties of the aetiology of bovine farcy isolated from
256 Sudanese cattle. Rev Elev Med Vet Pays Trop. 1979; 32: 135-41.

- 257 13. Cowan ST. ed. Manual for the Identification of Medical Bacteria, 2nd Edit.,
258 Cambridge University Press, London. 1974.
- 259 14. Isenberg HD. ed., Essentials Procedures for Clinical Microbiology.
260 Washington DC: ASM Press, pp.171–359. 1998.
- 261 15. Soddell JA, Stainsby FM, Eales KL, Seviour RJ, Goodfellow M. *Gordonia*
262 *defluvii* sp. nov., an actinomycete isolated from activated sludge foam. Int J
263 Syst Evol Microbiol. 2006; 56 (Pt 10): 2265-9.
- 264 16. Quinn PJ, Carter ME, Markey BK, Carter GR. The Actinomycetes. In:
265 Clinical Veterinary Microbiology, Quinn, P.J. (Eds.). Wolfe, Baltimore, pp:
266 144-155. 1999.
- 267 17. Isik K, Chun J, Hah YC, Goodfellow M. *Nocardia salmonicida* nom. rev., a
268 fish pathogen. Int J Syst Bacteriol. 1999; 49 Pt 2: 833-7.
- 269 18. Stanek JL, Roberts GD. Simplified approach to identification of aerobic
270 actinomycetes by thin-layer chromatography. Appl Microbiol. 1974; 28: 226-
271 31.
- 272 19. Hamid ME, Minnikin DE, Goodfellow M, Ridell M. Thin-layer
273 chromatographic analysis of glycolipids and mycolic acids from
274 *Mycobacterium farcinogenes*, *Mycobacterium senegalense* and related taxa.
275 Zentralbl Bakteriell. 1993; 279: 354-67.
- 276 20. Rainey FA, Ward-Rainey N, Kroppenstedt RM, Stackebrandt E. The genus
277 *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct
278 actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. Int J Syst
279 Bacteriol. 1996; 46: 1088-92.
- 280 21. Maidak BL, Cole JR, Parker CT Jr, Garrity GM, Larsen N, Li B, Lilburn TG,
281 McCaughey MJ, Olsen GJ, Overbeek R, Pramanik S, Schmidt TM, Tiedje
282 JM, Woese CR. A new version of the RDP (Ribosomal Database Project).
283 Nucleic Acids Res. 1999; 27: 171-3.
- 284 22. De Ley J, Cattoir H, Reynaerts A. The quantitative measurement of DNA
285 hybridization from renaturation rates. Eur J Biochem. 1970; 12: 133-42.
- 286 23. Huss VA, Festl H, Schleifer KH. Studies on the spectrophotometric
287 determination of DNA hybridization from renaturation rates. Syst Appl
288 Microbiol. 1983; 4: 184-92.
- 289 24. Cashion P, Holder-Franklin MA, McCully J, Franklin M. A rapid method for
290 the base ratio determination of bacterial DNA. Anal Biochem. 1977; 81: 461-
291 6.
- 292 25. Maldonado LA, Stainsby FM, Ward AC, Goodfellow M. *Gordonia sinesedis*
293 sp. nov., a novel soil isolate. Antonie Van Leeuwenhoek. 2003; 83: 75-80.
- 294 26. Stackebrandt E, Smida J, Collins MD. Evidence of phylogenetic
295 heterogeneity within the genus *Rhodococcus*: revival of the genus *Gordona*
296 (Tsukamura). J Gen Appl Microbiol. 1988; 34: 341–348.
- 297 27. Wayne LG. International Committee on Systematic Bacteriology:
298 announcement of the report of the *ad hoc* Committee on Reconciliation of

299 Approaches to Bacterial Systematics. Zentralbl Bakteriol Mikrobiol Hyg A.
300 1988; 268: 433-4.

301 28. Anderson PA, Petty BD. Mixed metazoan and bacterial infection of the gas
302 bladder of the lined seahorse-a case report. J Aquat Anim Health. 2013; 25:
303 42-52.

304 29. Sulieman MS, Hamid ME. Identification of acid fast bacteria from caseous
305 lesions in cattle in Sudan. J Vet Med B Infect Dis Vet Public Health. 2002;
306 49: 415-8.

307 30. Hamid ME. Epidemiology, pathology, immunology and diagnosis of bovine
308 farcy: a review. Prev Vet Med. 2012; 105: 1-9.

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