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Characterization of *Gordonia sinesedis* Isolated from a Zebu Cow Suffering from Lymphadenitis

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Authors' contributions

This work was carried out in collaboration between all authors. Author MEH performed the identification and wrote the manuscript. Author MTM made the original isolation of the strain. Authors MME and MJ contributed to identification; whilst authors SME and MG had supervised the M.Sc. and Ph.D. projects of author s MTM and MEH respectively. All authors read and approved the final manuscript

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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 bacterium, 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

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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.

1. INTRODUCTION

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

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

Microbiologic diagnosis of fastidious actinomycetes such as *Gordonia* spp. is often difficult and challenging. Consequently, significant numbers of misdiagnoses or incorrect identifications of clinical isolates may occur. Identification at the genus and species levels is presently obtained by 16S rRNA sequence comparisons [11].

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

2. MATERIALS AND METHODS

2.1 The Case

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

2.2 Isolation

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

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

2.3 Phenotypic Identification

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

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

2.4 Cell Wall Analysis

The isomeric form of diaminopimelic acid (meso-DAP) was determined by thin-layer chromatography (TLC) of whole-organism hydrolysates following the procedure described by Staneck and Roberts [18]. Standard procedures were also used for the extraction and analysis of mycolic acids [19], with the appropriate marker strains used as controls. The presence of single-spot co-chromatographs with reference *G. bronchialis* N654 (DSM 43247^T), confirmed 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 Master Pure^{TM} 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 CEQ^{TM}DTCS-Quick Start Kit (Beckmann Coulter) as directed in the manufacturer's protocol. Sequence reactions were electrophoresed using the CEQ^{TM}8000 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 Gen Bank database (http://www.ncbi.nlm.nih.gov/nucleotides).

2.6 DNA-DNA Hybridization

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

3. RESULTS

3.1 Isolation and Phenotypic Characterization

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

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

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].

3.2 Cell Wall Analysis

On thin layer chromatography the organism contained mycolic acids that co-chromatographed with mycolates from *Gordonia* species (Fig. 2).

3.3 16S rRNA Gene Sequence Analysis

Comparison of the complete 16S rDNA sequences (1517 bp; accession number: KC895879) of the isolate with corresponding nucleotide sequences of representatives of the suborder *Corynebacterineae* showed that the isolate belongs to the genus *Gordonia* (Fig. 3). It shares the highest 16S rRNA gene sequence similarity with *G. sinesedis* DSM 44455^T (100%), *Gordonia bronchialis* DSM 43297^T (97.2), *Gordonia polyisoprenivorans* DSM 44302^T (97.1%), *Gordonia araii* IFM 10211^T (97.0%); *Gordonia soli* CC-AB07^T (97.0%) and *Gordonia terrae* DSM 43249^T (97.0%).

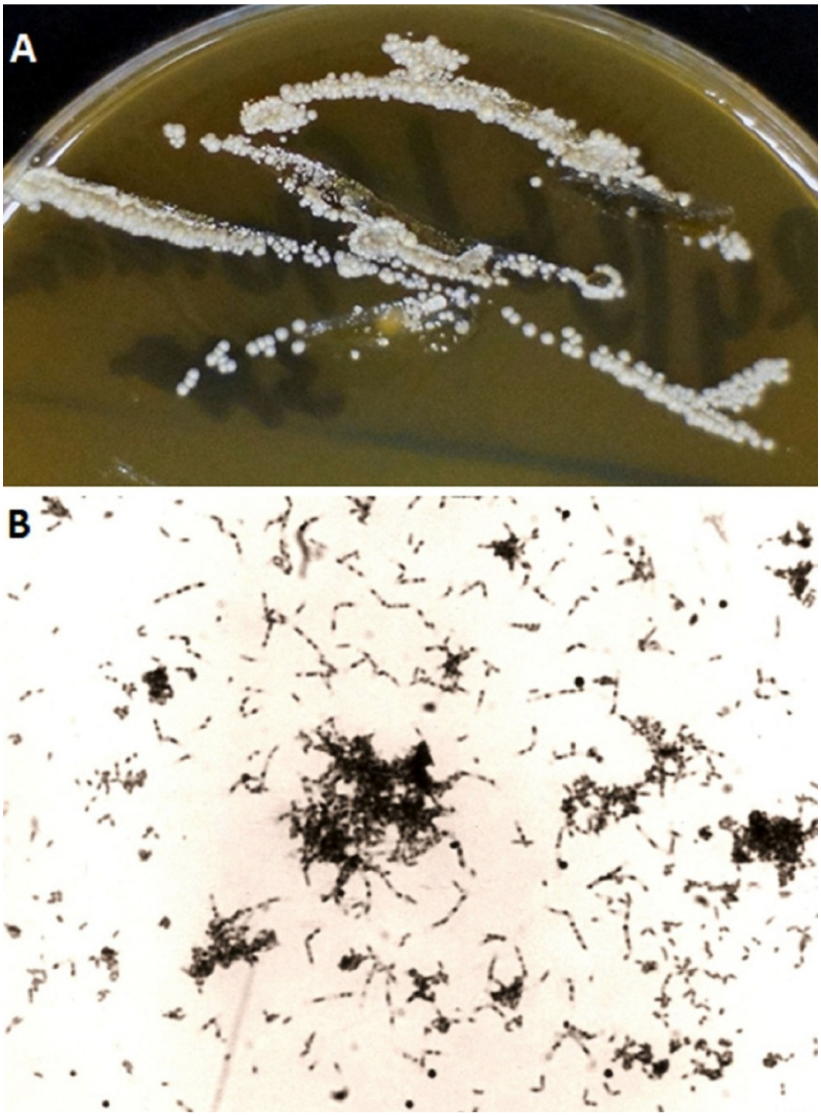

Fig. 1. Colony morphology of *Gordonia* sp. strain SD256 showing the growth of creamy, rough colonies with irregular edges (top), and microscopic picture of strain SD256 showing branching filaments (partially acid alcohol fast) which tends to break into short chains of coccobacillary elements (bottom)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

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

Species 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. rhizosphera 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 yellow**	**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)	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+

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

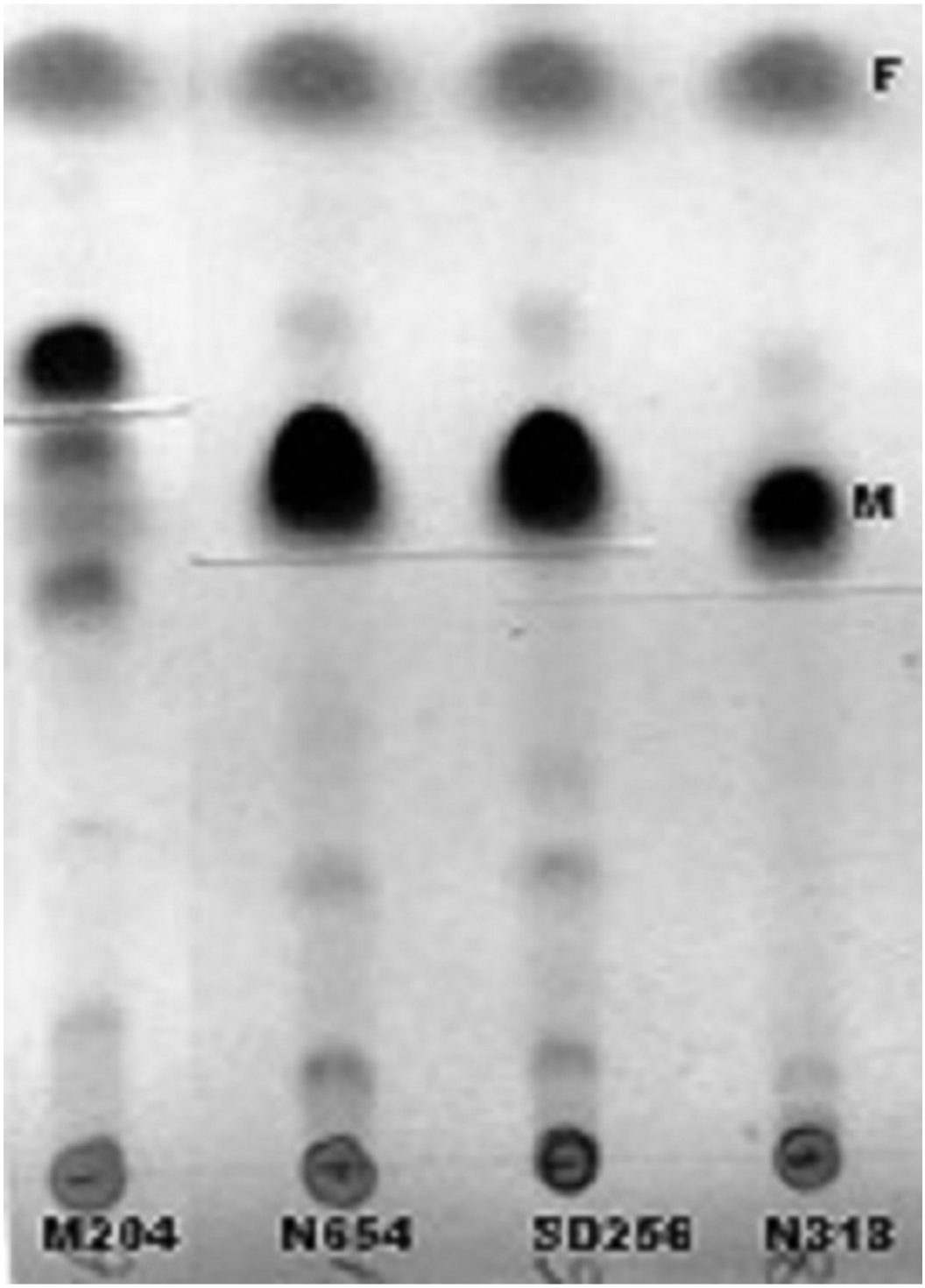

Fig. 2. One-dimensional TLC of methyl mycolates of *Gordonia* sp. (SD256) in comparison to *Gordonia bronchialis* (N654), *Nocardia brasiliensis* (N318) and *Mycobacterium fortuitum* (M204). Developing solvent: Petroleum ether (b.p. 60-80 "C)/diethyl ether (85: 15, v/v, x 2). Detection agent, 5% ethanolic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

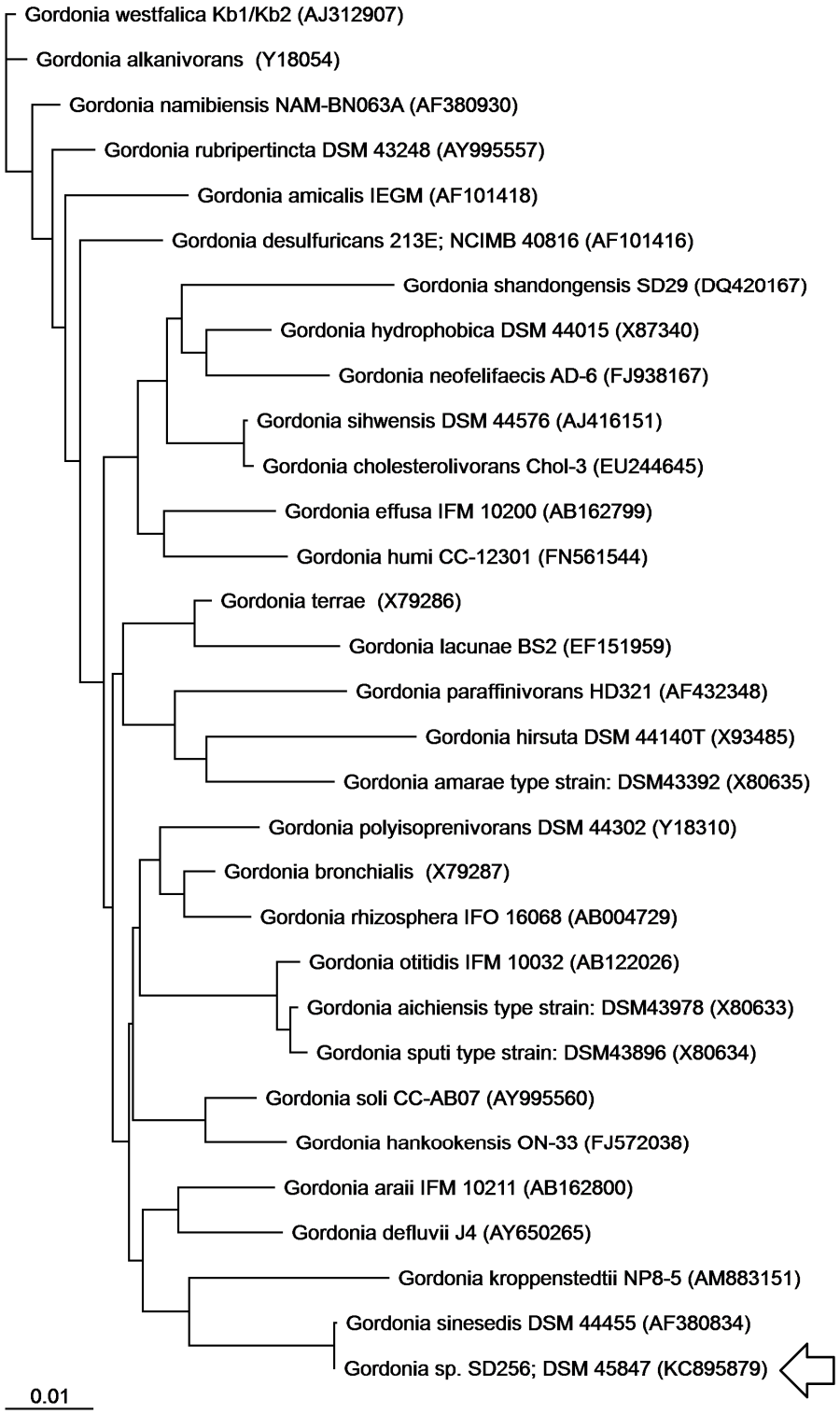

Fig. 3. Phylogenetic tree based on sequences derived from 16S rDNA gene showing the relationship between isolate (SD256; accession, KC895879) and members of the genus *Gordonia* and related mycolic acid containing actinomycetes

3.4 DNA-DNA Hybridization

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

4. DISCUSSION

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

The isolate SD256 was found to have cultural and morphological properties simulating those of mycolic acid-containing actinomycetes. Strain SD256 produced short, elementary branching hyphae (Fig. 1) that disintegrated into rod/coccus-like elements, showing the typical rod–coccus growth cycle that is usually found among strains of the genus *Gordonia* and related genera notably *Nocardia, Rhodococcus* and *Tsukamurella* [8]. Most of the morphological, physiological and chemotaxonomic characteristics of strain SD256 were coherent with the diagnostic features of the genus *Gordonia* [8,26]. It is evident from the results in Table 1 that the strain SD256 showed some phenotypic variations from *G. sinesedis*. But, 16S rDNA sequencing and DNA:DNA pairing revealed 100% and 99.6% similarity with *G. sinesedis*, respectively. According to the DNA:DNA pairing *Gordonia* sp. strain SD256 belongs to the species *Gordonia sinesedis* DSM 44455T. This assignment follows recommendations of a threshold value of 70% DNA-DNA similarity for the definition of bacterial species by the *ad hoc* committee [27].

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

5. CONCLUSION

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

COMPETING INTERESTS

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

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