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CASE REPORTS

Arthrobacter scleromae sp. nov. Isolated from Human Clinical Specimens

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A gram-positive, coryneform bacterium was isolated from swollen scleromata of a dermatosis patient. An analysis of its phenotypic, chemotaxonomic, and genotypic characteristics showed that this bacterium is closely associated with *Arthrobacter oxydans* and *Arthrobacter polychromogenes* but that it belongs to a distinct species, for which the name *Arthrobacter scleromae* sp. nov. is proposed.

CASE REPORT

A 19-year-old male presented to our clinic with a 2-month history of swollen scleromata on his back and hip. He complained of itching and pain at the foci of infection, especially when being touched, and of difficulties sitting and sleeping. No other relevant symptoms were present. While the patient was somewhat thin, his nutritional state was normal. He denied having any other previous illnesses of note or a family history of skin sensitivity.

Scattered, dull red, swollen dermal scleromata on the patient's back and hip were noted upon examination. Some of the scleromata were as large as chicken eggs, had white pus on the tops, and oozed a bloody effusion when pierced. No lymphadenopathy was detected, and the physical examination was otherwise normal. Routine hematological investigations revealed a leukocyte count of 6.5×10^9 cells/liter, with 38.5% lymphocytes, a hemoglobin level of 12.6 g/dl, and a platelet count of 250×10^9/liter. The lymphocyte T4/T8 ratio was 1.5. The serum immunoglobulin G (IgG) level was 1,120 mg/dl, the IgA level was 410 mg/dl, and the IgM level was 183 g/dl. Complement C3 was at 0.9 g/liter, C4 was at 0.75 g/liter, and CH50 was at 66.6 U. The serum HB_sAg test was negative. The scleromata developed slowly and looked like granuloma. No skin biopsy was performed. Samples of the bloody effusion were sent for bacterial, mycobacterial, and fungal cultures. A rapidly growing bacterium with numerous colonies in a pure culture state on blood agar was seen after 24 h at 35°C. The isolate was suspected of being *Staphylococcus epidermidis* due to colonial and cell morphology. But in succedent liquid culture, a rod coccus growth cycle was observed within 24 h at 35°C. Thus, a gram-positive coryneform bacterium, which was subsequently identified as an *Arthrobacter* sp., was recognized. The patient was treated with oral Chinese herbal medicine for heat clearing and detoxifying once a week for 1 month. Meanwhile, empirical antimicrobial therapy with oral ampicillin was initiated. Once the culture result was ascertained, on the fifth day from the start of the therapy, antibiotic treatment was changed to intravenous ceftriaxone and cefazolin for 2 weeks and then to oral ceftriaxone, with slow resolution of the lesion over a 2-month period.

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The genus *Arthrobacter* includes a heterogeneous group of aerobic, gram-positive, catalase-positive, nonfermentative coryneform bacteria of high G+C DNA content (18). Members of this genus contain L-lysine as the diamino acid in their cell walls and major cellular fatty acids (CFAs) of the branched type (11) and are divided into two groups, the A3α and A4α variations, based on their peptidoglycan structure (28). *Arthrobacter* spp. are widely distributed in the environment, especially in soil, and have recently been recognized as opportunistic pathogens. Among the 38 validly described *Arthrobacter* species at the time of writing of the present report, 5 were isolated from clinical sources, namely *Arthrobacter albus* (30), *Arthrobacter creatinolyticus* (13), *Arthrobacter cumminsii* (10), *Arthrobacter luteolus* (30), and *Arthrobacter woluwensis* (10). In addition, some strains of *Arthrobacter oxydans* were isolated from human blood (30). However, knowledge of the distribution, pathogenic potential, and clinical significance of *Arthrobacter* strains is far from adequate. The discovery of a new *Arthrobacter* species of human origin is therefore still significant. In this investigation, we report the characteristics of an unknown *Arthrobacter* species isolated from clinical specimens. On the basis of phenotypic, chemotaxonomic, and genotypic data, we propose the description of a new species of the genus *Arthrobacter*, *Arthrobacter scleromae*.

Strain YH-2001^T (AS 1.3601^T; China General Microbiological Culture Collection Center, Chinese Academy of Sciences)

was isolated from the bloody effusion from swollen dermal scleromata of the patient. Additionally, two reference strains, *Arthrobacter oxydans* AS 1.1925^T (DSM 20119^T) and *Arthrobacter polychromogenes* AS 1.1927^T (DSM 20136^T), were included for comparison in this study. These organisms were inoculated on nutrient agar and incubated at 30°C.

The morphology and motility of cells were examined by using a model FEI QUANTA electron microscope and a Zeiss Axioskop 20 light microscope. Motility was tested by the hanging-drop technique (29). Flagellum staining was performed as described by Kodaka et al. (19). Biochemical tests, including utilization of carbohydrates as sole carbon sources, were performed by using either conventional methods (11, 12) or commercially available API Coryne and API 50 CH kits (bioMérieux, Marcy l'Étoile, France) according to the manufacturer's instructions.

Antibiotic susceptibility was determined by the E test on Mueller-Hinton blood agar incubated at 35°C for 24 h. The results were interpreted according to the criteria established for staphylococci in 1997 by the National Committee for Clinical Laboratory Standards (24).

Cell-wall diamino acids and sugars were prepared and analyzed as described by Komagata and Suzuki (21). Detailed analysis of the peptidoglycan structures was performed by following the methods of Schleifer and Kandler (28). The amino acids and peptides were separated by two-dimensional ascending thin-layer chromatography on cellulose sheets (Merck) by using the solvent systems described by Schleifer and Kandler (28). Menaquinones were extracted from freeze-dried biomass, purified according to the method of Collins (4), and then analyzed by high-pressure liquid chromatography. For analysis of CFAs, cells of the test strain were cultured on trypticase soy agar (BBL) for 48 h at 30°C. CFAs were extracted, methylated, and analyzed by gas chromatography by using the standard Microbial Identification System (15). The base composition of the genomic DNA of the strain was determined by the thermal denaturation method (23) with *Escherichia coli* AS 1.365 as a control.

Genomic DNA preparation and PCR amplification of the 16S rRNA gene were performed by using the method of Chun and Goodfellow (3). The amplified product was sequenced as described previously (14). The nucleotide sequence was obtained automatically by using an Applied Biosystems DNA sequencer (model 377) and software provided by the manufacturer. The 16S rRNA gene sequence of strain YH-2001^T was aligned manually by using the CLUSTAL X 1.8 program against corresponding sequences retrieved from the GenBank database. Phylogenetic trees were inferred by using the neighbor-joining (26), least-squares (9), and maximum-likelihood (6) treeing algorithms from the PHYLIP 3.5c suite of programs (8). Evolutionary distance matrices were generated by the method of Kimura (16). The resultant unrooted tree topologies were evaluated by bootstrap analyses (7) of the neighbor-joining method based on 1,000 resamplings with the SEQBOOT and CONSENSE programs in the PHYLIP package.

Hybridizations between genomic DNA of strain YH-2001^T and that of its nearest neighbors were carried out by following the micro-well method (2, 5) with a FLUOstar OPTIMA microplate reader (BMG LABTECH, Offenburg, Germany) for the fluorescence measurements. Single-stranded unlabeled DNA was noncovalently bound to microplate wells. Hybridizations were performed at 47°C overnight in a hybridization solution containing 50% formamide but no dextran sulfate. Salmon sperm DNA was used as the negative control in all experiments. Each experiment was performed with four replicates. The percentage of DNA homology was calculated as described by Christensen et al. (2). DNA-DNA homology data generated in this study are presented as means of reciprocal values. The DNA homology value in individual experiments never showed more than 10% deviation from the mean.

Strain YH-2001^T consisted of gram-positive, non-spore-forming, nonmotile, coryneform cells, which displayed a typical rod-coccus life cycle. It was aerobic, catalase-positive, and nonfermentative, producing white, glistening, circular colonies with a smooth convex surface. The colonies were able to grow to up to 5.0 mm in diameter after a 72-h incubation. The strain contained L-lysine as the diamino acid in its cell wall and a predominant amount of anteiso-$C_{15:0}$ as the major CFA, but it lacked mycolic acids. These general properties were consistent with members of the genus *Arthrobacter*. Results from API test systems showed that the organism was positive for gelatin liquefaction, casein, DNA, esculin, starch, and tyrosine hydrolysis; weakly positive for nitrate reduction; and negative for *N*-acetylglucosaminidase, lecithinase, lipase, and urease. No acid was produced from the carbohydrates tested, except mannitol when grown aerobically. The organism assimilated L-arginine, L-asparagine, citrate, D-fructose, D-galactose, D-gluconate, D-glucose, glycerol, L-histidine, lactose, maltose, mannose, D-melezitose, pyruvate, D-raffinose, sorbitol, sucrose, trehalose, xylitol, and D-xylose.

The isolate possessed a peptidoglycan of type A3α with an L-Lys–L-Ser–L-Thr–L-Ala interpeptide bridge, which is found in four members of the genus *Arthrobacter*, i.e., *A. chlorophenolicus*, *A. oxydans*, *A. polychromogenes*, and *A. sulfonivorans* (1, 20, 32). However, strain YH-2001^T had a markedly different isoprenoid quinone profile, composed of a major amount of MK-8(H_2) and a minor amount of MK-10 (peak area ratio, 88:12). It was also easily distinguished from *A. chlorophenolicus* and *A. sulfonivorans* by colony color, motility, and growth ability at 5°C. A comparison between the chemotaxonomic and phenotypic characteristics of strain YH-2001^T and those of the four *Arthrobacter* species mentioned above is shown in Table 1.

To further clarify the relationships between this unknown bacterium and the recognized *Arthrobacter* species, its almost complete 16S rRNA gene sequence (1,415 nucleotides) was determined and compared with those of representative reference strains of the genus *Arthrobacter*. The result clearly showed that the organism belongs to this genus. In accordance with the result of cell-wall peptidoglycan analysis, the bacterium formed a closely associated clade with *A. oxydans* and *A. polychromogenes*, supported by a high bootstrap value of 100% (Fig. 1). High 16S rRNA gene sequence similarities of 99.4 (*A. oxydans* DSM 20119^T) and 99.3% (*A. polychromogenes* DSM 20136^T) were found between the test strain and its two nearest neighbors. But this result did not indicate that they belong to the same genospecies, as higher 16S rRNA gene sequence similarities were recorded between representatives of several well-established *Arthrobacter* species, for example, 99.7% between *Arthrobacter nitroguajacolicus* and *Arthrobacter aurescens* (22) and 100% between *Arthrobacter pascens* and *Arthrobacter*

TABLE 1. Comparison of characteristics of *Arthrobacter* species with Lys-Ser-Thr-Ala-type peptidoglycan[a]

Characteristic	Result for organism indicated				
	A. scleromae	*A. chlorophenolicus*	*A. oxydans*	*A. polychromogenes*	*A. sulfonivorans*
Cell-wall sugars	Gal, Glu	ND	Gal, Glu	Gal	ND
Major menaquinone	MK-8(H_2)	ND	MK-9(H_2)	MK-9(H_2)	MK-9(H_2)
CFA composition (%):					
i-$C_{14:0}$	1	4	2	1	1
$C_{14:0}$	1	2	2	1	1
i-$C_{15:0}$	11	7	9	3	15
ai-$C_{15:0}$	50	66	45	44	63
$C_{15:0}$	−	−	1	1	−
i-$C_{16:1}$	4	1	−	−	−
i-$C_{16:0}$	7	10	11	6	6
$C_{16:1}\omega7c$	5	1	−	−	−
$C_{16:0}$	2	3	11	13	4
i-$C_{17:1}\omega9c$	2	−	−	−	−
ai-$C_{17:1}\omega9c$	7	1	−	−	−
i-$C_{17:0}$	1	−	2	1	1
ai-$C_{17:0}$	8	5	17	25	7
G+C content of DNA (mol%)	64.7	65.1	63.1	62.9	61
Colony color	White	Grey	Grey-white	Blue-green	Creamy yellow
Motility	−	+	−	−	+
Growth at:					
5°C	−	+	−	−	+
37°C	+	+	+	+	−

[a] Data were taken from the present study, Borodina et al. (1), Kodama et al. (20), Reddy et al. (25), and Westerberg et al. (32). +, positive or present; −, negative or absent; ND, not determined.

ramosus (17). Actually, strain YH-2001^T exhibited a number of characteristics distinct from its nearest phylogenetic relatives (Table 2). Despite the fact that some strains of *A. oxydans* were also isolated from human clinical specimens (30), strain YH-2001^T differed from *A. oxydans* by lacking β-galactosidase, α-glucosidase, and the capability to grow on nicotine and on 10% NaCl. The API Coryne code for the strain was 1042014, which was identified as a doubtful or unacceptable profile for *Rhodococcus* spp. in the manufacturer's database, whereas the API Coryne code for *A. oxydans* was 3750004 (*Arthrobacter* spp.) (30). *A. polychromogenes* was isolated from air, and it forms blue-pigmented colonies on carbohydrate-peptone medium (27), a feature distinguishing it from the other *Arthrobacter* species and from strain YH-2001^T as well. Furthermore, unlike *A. oxydans* and *A. polychromogenes*, the test strain could not utilize 2,3-butanediol, formate, L-leucine, malonate, L-rhamnose, or D-ribose as the sole carbon source, nor could it produce acid from glucose, but it was able to grow in mineral salt medium with ammonium or nitrate as the sole nitrogen source, while *A. oxydans* and *A. polychromogenes* required biotin (32).

DNA-DNA reassociation values between strain YH-2001^T and *A. oxydans* DSM 20119^T and *A. polychromogenes* DSM 20136^T were 51 and 36%, respectively. These values are well below the 70% threshold for delineation of genomic species (31), thereby underpinning the idea that the unknown isolate should be assigned to a separate species. On the basis of phenotypic, chemotaxonomic, phylogenetic, and DNA-DNA hybridization evidence, strain YH-2001^T merits a new species status in the genus *Arthrobacter*, for which we propose the name *Arthrobacter scleromae* sp. nov. Since it was isolated from several cultures of bloody effusion from dermal scleromata of a patient and numerous colonies in a pure state were present at primary isolation, it was not considered to be an environmental contaminant. We believe that the formal description of this new *Arthrobacter* species will facilitate its recognition in the clinical laboratory and contribute to a better understanding of the role of *Arthrobacter* strains in human disease.

Description of *Arthrobacter scleromae* sp. nov. *Arthrobacter scleromae* (scle′ro.mae. L. adj. *scleromae*, of scleroma) cells are gram-positive, non-spore-forming, and nonmotile and display a rod-coccus life cycle. They are obligately aerobic, catalase-positive, and ~0.25 to ~0.35 μm in diameter. Colonies on blood agar or nutrient agar are whitish, glistening, convex, smooth surfaced, and circular. The colonies grow to up to 4 to 5 mm in size by 72 h. Growth occurs with a suitable carbon source in mineral salt medium; no additional growth factors are needed. Growth also occurs in the presence of 5% NaCl, at between 15 and 37°C and between pHs 6 and 9, but not on 10% NaCl, at 5 or 42°C. The organism hydrolyzes casein, DNA, esculin, gelatin, starch, and tyrosine, but not lecithin or xanthine. The nitrate reduction test is weakly positive. *N*-acetylglucosaminidase, β-galactosidase, α-glucosidase, lipase, pyrrolidonyl peptidase, and urease are not produced. Acid is produced from mannitol. It utilizes the following substrates as sole carbon sources: γ-aminobutyrate, L-arginine, L-asparagine, citrate, D-fructose, fumarate, D-galactose, D-gluconate, D-glucose, glycerol, L-histidine, *p*-hydroxybenzoate, 2-ketoglutarate, lactose, DL-malate, maltose, mannose, D-melezitose, phenylacetate, pyruvate, D-raffinose, salicin, sorbitol, succinate, sucrose, trehalose, D-turanose, xylitol, and D-xylose. The following substrates are not utilized: acetamide, adipate, adonitol, L-alanine, *p*-aminobenzoate, D-arabitol, arbutin, azelate, benzoate, 2,3-butanediol, *n*-butyrate, caprylate, D-cellobiose, L-cit-

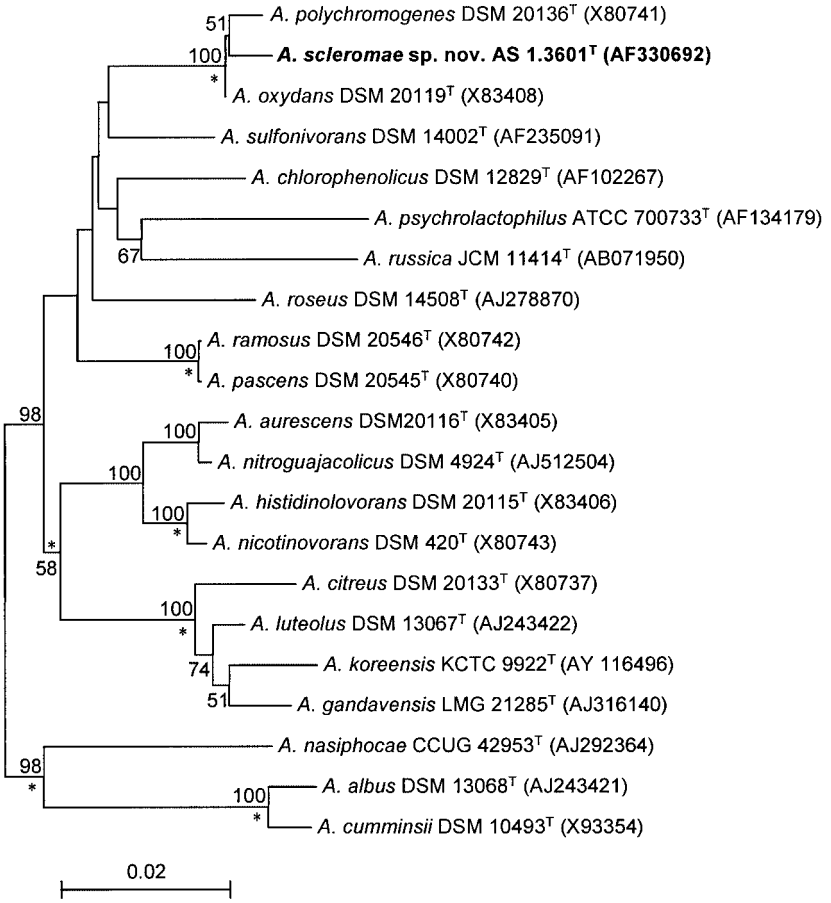

FIG. 1. Unrooted neighbor-joining tree showing the phylogenetic position of *A. scleromae* sp. nov. AS 1.3601^T within the genus *Arthrobacter*. Asterisks indicate branches that were also recovered by using the least-squares and maximum-likelihood methods. The numbers at the nodes indicate the levels of bootstrap support (%) based on a neighbor-joining analysis of 1,000 resampled data sets; only the values over 50% are given. The scale bar indicates 0.02 substitutions per nucleotide position.

rulline, L-cysteine, dulcitol, erythritol, formate, glucosamine, D-glucuronate, glutarate, glycogen, glycolate, γ-hydroxybutyrate, inulin, DL-isoleucine, isovalerate, L-leucine, maleate, malonate, DL-methionine, α-*methyl*-D-glucoside, α-*methyl*-D-mannoside, nicotine, L-ornithine, oxalate, L-phenylalanine, *o*-phthalate, pimelate, L-rhamnose, D-ribose, sebacate, sorbose, suberate, D-tartrate, L-threonine, L-tryptophan, or DL-valine. Compounds slowly utilized are acetate, L-arabinose, inositol, mannitol, and melibiose. The strain is susceptible to ceftriaxone, chloramphenicol, rifampin, and tetracycline; moderately susceptible to cefazolin, cefotaxime, doxycycline, erythromycin, nitrofurantoin, piperacillin, and vancomycin; and resistant to amikacin, ampicillin, gentamicin, kanamycin, norfloxacin, oxacillin, penicillin G, streptomycin, and tobramycin. The DNA G+C content is 64.7 mol%. The major CFA is anteiso-$C_{15:0}$, with significant amounts of i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:1}\omega9c$,

TABLE 2. Biochemical characteristics distinguish *Arthrobacter scleromae* from its nearest phylogenetic relatives[a]

Species	Results with:								Utilization of:								
	Nic	NO_3	10% NaCl	Sta	Vit	Glu	β-Gal	α-Glu	L-Leu	L-Ara	L-Rha	D-Rib	2,3-But	Ino	Mal	For	Pro
A. oxydans	+	+	+	+	+	+	+	+	W	+	+	+	W	W	+	+	+
A. polychromogenes	−	+	−	W	+	+	ND	ND	W	+	+	+	W	−	+	+	+
A. scleromae	−	W	−	+	−	−	−	−	−	W	−	−	−	W	−	−	W

[a] Data were taken from this study, Kodama et al. (20), and Wauters et al. (30). +, positive or present; W, weak positive; −, negative or absent; ND, not determined; Nic, utilization of nicotine; NO_3, nitrate reduction; 10% NaCl, growth on 10% (wt/vol) NaCl; Sta, hydrolysis of starch; Vit, vitamin requirement; Glu, acid from glucose; β-Gal, β-galactosidase; α-Glu, α-glucosidase; L-Leu, L-leucine; L-Ara, L-arabinose; L-Rha, L-rhamnose; D-Rib, D-ribose; 2,3-But, 2,3-butanediol; Ino, inositol; Mal, malonate; For, formate; Pro, propionate.

and ai-$C_{17:0}$. The predominant menaquinone is MK-8(H_2). The cell-wall peptidoglycan type is L-Lys–L-Ser–L-Thr–L-Ala (A3α), and the cell-wall sugars are galactose and glucose. It was isolated from swollen scleromata of a dermatosis patient. The type strain is AS 1.3601^T.

Nucleotide sequence accession number. The GenBank accession number for the 16S rRNA gene sequence of *Arthrobacter scleromae* AS 1.3601^T is AF330692.

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