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Cellulosimicrobium arenosum sp. nov., Isolated from Marine Sediment Sand

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Abstract

A Gram-stain-positive, non-spore-forming, yellow pigmented, non-motile, aerobic, short rod-shaped bacterial strain, designated CAU 1455^T, was isolated from marine sediment sand. Strain CAU 1455^T grew optimally at 30 °C and at pH 7.5 in the presence of 1% (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain CAU 1455^T was affiliated to the genus *Cellulosimicrobium* and was most closely related to *Cellulosimicrobium terreum* DS-61^T (similarity 97.9%). The strain possessed MK-9 (H_4) as the predominant menaquinone and anteiso-$C_{15:0}$ as the major cellular fatty acids. Peptidoglycan type was A4a (L-Lys–D-Glu2). The DNA G+C content was 74.3 mol% and the level of DNA–DNA relatedness between CAU 1455^T and *C. terreum* DS-61^T was 27.8%. Based on phenotypic, chemotaxonomic, and genetic data, strain CAU 1455^T represents a novel species of the genus *Cellulosimicrobium*, for which the name *Cellulosimicrobium arenosum* sp. nov. is proposed. The type strain is CAU 1455^T (= KCTC 49039^T = NBRC 113062^T).

Introduction

The genus *Cellulosimicrobium*, a member of the family *Promicromonosporaceae* was proposed by Schumann et al. [20] with reclassification of *Cellulomonas cellulans* as *Cellulosimicrobium cellulans*, the type species of the genus. Currently, this genus comprises five recognized species with validly published names [17]. Members of this genus are catalase-positive, Gram-stain-positive short rod or coccoid bacteria that have a high DNA G+C content, type A4α as a major cell-wall peptidoglycan, and MK-9 (H_4) as the predominant menaquinone [3, 11, 22, 25]. Members of the genus *Cellulosimicrobium* have been isolated from various environment such as human blood [3], soil [25], sea sediment [11], and freshwater [22]. The novel bacterial strain, designated CAU 1455^T, was isolated from a marine sediment sand sample collected in Modo Island (37°31′52.4″N 126°24′42.3″E) in the Republic of Korea. Phenotypic, chemotaxonomic, and phylogenetic characteristics suggested that it represents a novel species of the genus *Cellulosimicrobium*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAU 1455T is MG214548.

The digital protologue database (DPD) number of Current Microbiology for the strain CAU 1455T is TA00408.

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Materials and Methods

Isolation of Bacterial Strain and Culture Condition

Strain CAU 1455^T was isolated from a marine sand sample according to Gordon and Mihm [9] on marine agar 2216 (MA; Difco, USA) plates. The crushed soil sample was serially diluted with sterilized 0.9% sodium chloride solution and plated on MA at 30 °C for 7 days. The type strains of the most closely related species, *Cellulosimicrobium terreum* KCTC 19206^T, *Cellulosimicrobium funkei* KCTC 39619^T, *Cellulosimicrobium marinum* NBRC 110994^T, *Cellulosimicrobium cellulans* KCTC 1771^T, and *Cellulosimicrobium*

aquatile KCTC 39527^T were obtained from the Korean Collection for Type Cultures (KCTC; Jeongeup, Korea) and the National Institute of Technology and Evaluation (NBRC; Chiba, Japan) were used as reference strains.

Phenotypic and Biochemical Characteristics

Strain CAU 1455^T was cultivated on MA at 30 °C to investigate phenotypic and biochemical characteristics [1]. The spore formation was observed on nutrient sporulation medium [16]. Cell morphology was examined by light microscopy (model DM 1000; Leica, Germany) and transmission electron microscopy (model JEM 1010, JEOL, Japan). Gram staining was tested using the bioMérieux Gram staining kit (bioMérieux, France). Gliding motility was evaluated according to a previously described method [2]. The temperature range for growth and relation to oxygen of strain CAU 1455^T in MA at 4, 10, 20, 30, 37, 45, and 55 °C in an aerobic incubator (model MIR-253; Sanyo, Japan) and in an anaerobic chamber (model Bactron; Sheldon, UK) was examined by measuring the turbidity of the marine broth 2216 (MB, Difco, USA) after 72 h. The pH ranges for growth were tested at 30 °C in MB adjusted to pH 4.5–11.0 at 0.5 pH unit intervals. The pH values of <6, 6–9, and >9 were obtained by using sodium acetate/acetic acid, Tris/HCl, and Na_2CO_3 buffers, respectively. Tolerance of NaCl was tested with NaCl concentrations at 0–15% (w/v) (at increments of 1% intervals) was examined at 30 °C in MB prepared according to the formula of the Difco medium except that NaCl was excluded. Catalase and oxidase activities were tested according to Cappuccino and Sherman [4]. Hydrolysis of casein, gelatin, esculin, and nitrate reduction were determined according to Smibert and Krieg [21]. Physiological and biochemical characteristics and acid production from carbohydrates were tested using the API 20E, API 50CH, and API ZYM systems (bioMérieux) according to the manufacturer's instruction.

16S rRNA Gene Sequencing and Phylogenetic Analysis

Genomic DNA of strain CAU 1455^T was extracted using a genomic DNA extraction kit (Intron, Korea). The amplification of 16S rRNA gene was conducted by PCR with the universal primers 8F/1525R following established procedures [14]. The amplicon of 16S rRNA gene was sequenced using a 3730 automatic DNA sequencer (Applied Biosystems, USA). Identification of the strain was performed based on 16S rRNA gene sequencing followed by pairwise similarity calculation between strain CAU 1455^T and other closely related strains using EzBioCloud database (http://www.ezbiocloud.net) [26]. Multiple alignments and calculation of sequence similarity levels with members of the genus *Cellulosimicrobium* and other closely related genera were performed by using the CLUSTAL_X 2.1 software [15]. The neighbor-joining [19], maximum-likelihood [6], and Fitch–Margoliash [8] (DNAml program from the PHYLIP 3.66 package) algorithms were used in phylogenetic tree building. The distance matrix was generated according to the Jukes–Cantor model [12]. The tree topology in the neighbor-joining phylogenetic tree was evaluated by the bootstrap resampling method [7] with 1,000 replicates with the SEQBOOT and CONSENSE programs from the PHYLIP package. HPLC. The extent of DNA–DNA relatedness between CAU 1455^T and the phylogenetically most closely related neighbor, *C. terreum* DS-61^T was examined using the fluorometric microplate method [5], as modified by Goris et al. [10]. The mol% G+C content of the genomic DNA was determined using HPLC by the method of Tamaoka and Komagata [23].

Chemotaxonomic Analysis

For cellular fatty acid analysis, the cell mass of strain CAU 1455^T and five reference strains of the genus *Cellulosimicrobium* were harvested from MA plate after cultivation for 3 days at 30 °C. The fatty acid methyl esters were prepared and extracted according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System [18]. Determination of the cell-wall peptidoglycan type of strain CAU 9143T was carried out by the Identification Service of the DSMZ (Braunschweig, Germany). Respiratory quinones were isolated and analyzed as described previously using HPLC [13].

Results and Discussion

Morphological and Phenotypic Characteristics

The morphological, phenotypic, and biochemical characteristics of strain CAU 1455^T are listed in the species description. Comparison of differential characteristics with closely related species is shown in Table 1. Growth of strain CAU 1455^T was observed at 35 °C, but *C. terreum* was not. This isolate grew in a pH range between 5.5 and 8.5 and the salinity range for growth was found to be 0–8% (w/v) NaCl. Phenotypic characteristics data of strain CAU 1455^T were distinct among the species of genus *Cellulosimicrobium* by its positive reaction for L-rhamnose, and negative reaction for *N*-acetylglucosamine, leucine arylamidase, and α-glucosidase. All negative results from the commercial test kits are indicated in Supplementary Table 1.

Table 1 Differential properties of strain CAU 1455^T and the type strains of the most closely related *Cellulosimicrobium* species

Characteristic	1	2	3	4	5	6
Motility	–	–[a]	+[b]	–[c]	–[d]	–[e]
Growth at 35 °C	+	–	+	+	+	+
pH range	5.5–8.5	6–9[a]	ND	6–11[c]	ND	6–9[e]
NaCl (%, w/v) range	0–8	0–9 [a]	ND	0–7[c]	ND	0–13[e]
Acid production from						
L-Rhamnose	+	–	–	–	–	–
N-acetylglucosamine	–	+	+	+	+	+
Utilization of:						
2-nitrophenyl-β D-galactopyranoside	–	+	+	+	+	+
D-Sucrose	–	+	+	+	+	+
Amygdalin	–	+	+	+	+	+
Enzyme activities						
Leucine arylamidase	+	–	–	–	–	–
α-Glucosidase	–	+	+	+	+	+
DNA G+C content (mol %)	74.3	72.9[a]	74.5[b]	75.6[c]	74[c]	73.8[e]

Strains 1 CAU 1455^T, 2 *C. terreum* KCTC 19206^T, 3 *C. funkei* KCTC 39619^T, 4 *C. marinum* NBRC 110994^T, 5 *C. cellulans* KCTC 1771^T, 6 *C. aquatile* KCTC 39527^T. Data were taken from this study unless indicated. All strains were positive for hydrolysis of aesculin. + positive, – negative, *ND* no data available

Data taken from: a Yoon et al. [25], b Brown et al. [3], c Hamada et al. [11], d Schumann et al. [20], e Sultanpuram et al. [22]

Phylogenetic and Genomic Characteristics

The almost-complete 16S rRNA gene sequence of strain CAU 1455^T (1457 bp) was determined. The neighbor-joining tree showed that the isolate falls within the genus *Cellulosimicrobium* and was most closely related to *C. terreum* DS-61^T with the 16S RNA gene sequence similarity of 97.9% (Fig. 1). The sequence similarity of strain CAU 1455^T and other species in the genus *Cellulosimicrobium* were 97.2–97.3%. The phylogenetic relatedness was found to be similar to the neighbor-joining tree in the maximum-likelihood, and Fitch–Margoliash algorithms (data not shown). The DNA–DNA relatedness between CAU 1455^T and *C. terreum* DS-61^T was 27.8%. The value is below the 70% cut-off point suggested by Wayne et al. [24] for the determination of genomic species, supporting the proposal that strain CAU 1455^T denotes a separate species. The G+C content of the DNA of strain CAU 1455^T was 74.3 mol%, which was close to the values observed for other species of the genus *Cellulosimicrobium*.

Chemotaxonomic Analysis

The major cellular fatty acids of strain CAU 1455^T were found to be anteiso-$C_{15:0}$ (48.5%) and anteiso-$C_{17:0}$ (25.9%). The fatty acid profile was similar to the reference type strains, but the proportion of some components was different from each other (Table 2). The total hydrolysates of the peptidoglycan contained the amino acids lysine (Lys), alanine (Ala), threonine (Thr), aspartic acid (Asp), and glutamic acid (Glu) in the molar ratio of approximately 0.7: 4.1: 1.8: 1.1: 1.0, and the partial hydrolysate of the peptidoglycan contained the peptides L-Ala–D-Glu, L-Lys–L-Thr, L-Lys–L-Thr–D-Ala, and L-Lys–D-Ala. From these data, the cell-wall peptidoglycan was of the type A4a L-Ala–D-Glu2. This interpeptide bridge was the same as those of *C. terreum* and *C. marinum* but differed from those of *C. cellulans* and *C. aquatile* [3, 11, 20, 25]. The tetrahydrogenated menaquinone with nine isoprene units [MK-9 (H4)] was detected as a predominant isoprenoid quinone in strain CAU 1455^T. This feature is compatible with the genus *Cellulosimicrobium*.

Taxonomic Conclusion

Phylogenetically, CAU 1455^T belongs to the genus *Cellulosimicrobium* and has characteristics similar to those of other *Cellulosimicrobium* species, as shown by anteiso-$C_{15:0}$ being the most abundant fatty acids. Therefore, phenotypic, genetic, and chemotaxonomic characteristics provide sufficient evidence to recognize strain CAU 1455^T as a novel species of the genus *Cellulosimicrobium* for which the name *Cellulosimicrobium arenosum* sp. nov. is proposed.

Description of Cellulosimicrobium Arenosum sp. Nov.

Cellulosimicrobium arenosum sp. nov. (a.re.no'sum. L. neut. adj. *arenosum* sandy, dwelling in marine sediment sand).

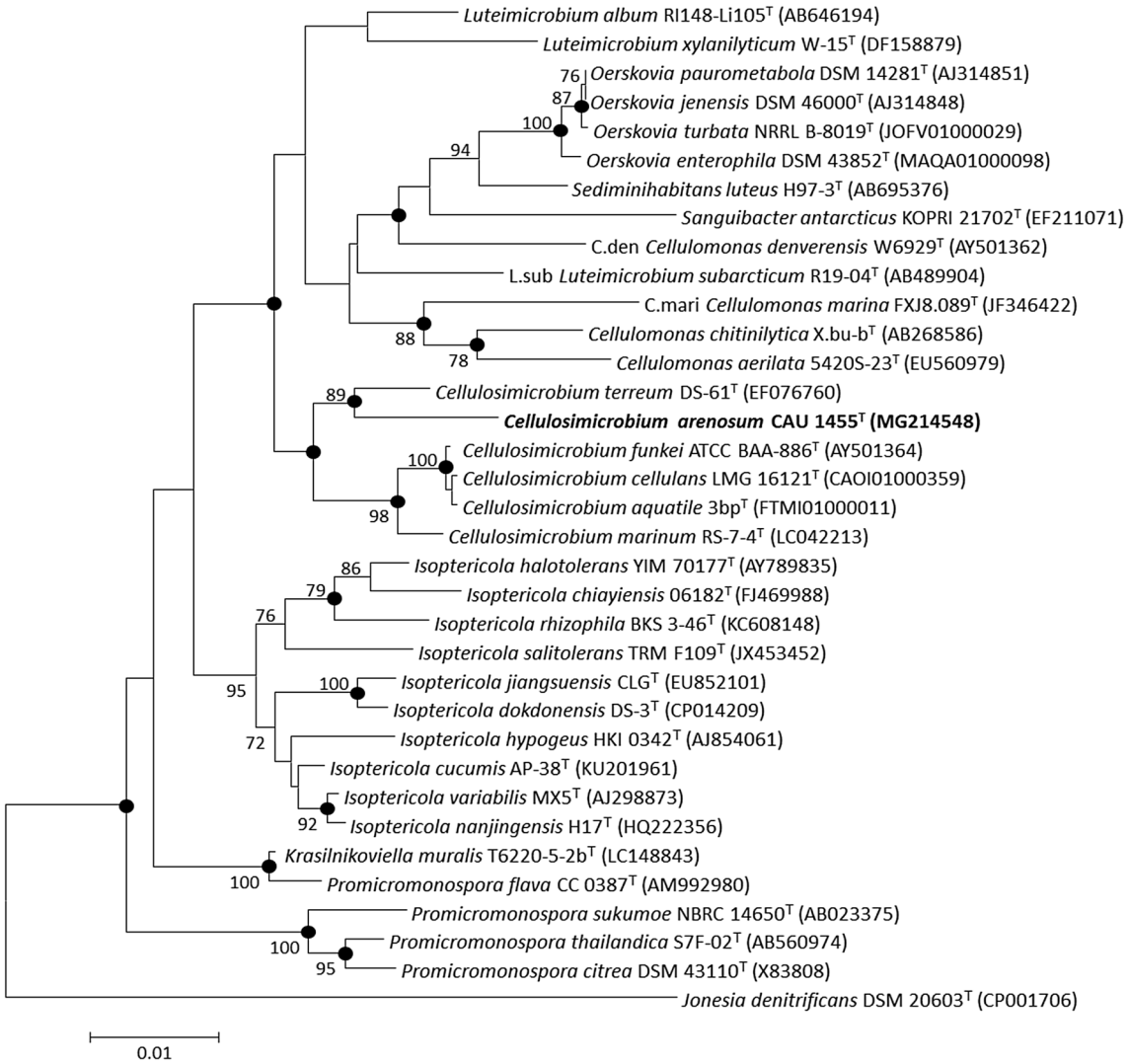

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain CAU 1455^T and the type strains of reference *Cellulosimicrobium* species. Dots indicate that the corresponding nodes were also recovered in the trees created with the maximum-likelihood and Fitch–Margoliash algorithms. Bootstrap values are indicated as percentages of 1000 resampled datasets, when greater than 70%. Bar, 0.01 substitutions per nucleotide position. *Jonesia denitrificans* DSM 20603^T (CP001706) is used as an outgroup organism

Cells are gram-stain-positive, non-motile, aerobic, non-spore-forming, and short rod-shaped bacterial strain, approximately 0.4–0.9 μm in diameter and 0.5–2.5 μm in length. Colonies grown on marine agar. after 3 days of incubation at 30 °C are yellow colored, circular with entire margins. Growth occurs at 20–37 °C (optimum, 30 °C), at pH 6.0–8.5 (optimum, 7.5), and with 0–8% (w/v) NaCl (optimum 1%). Catalase is present, and starch is liquefied. Strain CAU 1455^T utilizes D-glucose, L-arabinose, and trisodium citrate. Acid is produced from D-arabinose, L-arabinose, D-cellobiose, D-fructose, D-galactose, gentiobiose, methyl-α D-glucopyranoside, D-glucose, glycerol, glycogen, D-lactose, D-maltose, D-mannose, D-melezitose, D-melibiose, D-raffinose, L-rhamnose, D-saccharose, salicin, D-trehalose, D-turanose, xylitol, D-xylose Methyl-β, and D-xylopyranoside. Acid phosphatase, *N*-acetyl-β-glucosaminidase activities, and naphthol-AS-BI-phosphohydrolase activities are present. The major isoprenoid quinone is MK-9 (H_4). The main fatty acid of strain is anteiso-$C_{15:0}$ and the cell-wall peptidoglycan type is A4α L-Lys–L-Thr–D-Asp. The DNA G+C content is 74.3 mol%. The type strain CAU 1455^T (=KCTC 49039^T=NBRC 113062^T), isolated from marine sediment sand in Modo Island collected in the Republic of Korea.

Table 2 Cellular fatty acid compositions (%) of strain CAU 1455^T and the type strains of the most closely related *Cellulosimicrobium* species

Fatty acids	1	2	3	4	5	6
Saturated fatty acid						
$C_{14:0}$	1.8	2.3	3.5	4.2	3.1	2.3
$C_{16:0}$	6.6	4.8	6.0	7.1	6.1	5.0
Branched fatty acid						
iso-$C_{14:0}$	1.2	6.1	4.7	2.8	6.8	3.5
iso-$C_{15:0}$	7.0	9.7	12.0	12.0	10.4	9.9
anteiso-$C_{15:0}$	48.5	50.3	43.3	43.1	41.9	43.2
iso-$C_{16:0}$	7.1	12.7	18.6	16.3	23.1	20.9
anteiso-$C_{17:0}$	25.9	11.8	10.8	11.4	6.8	13.6
iso-$C_{17:0}$	1.1	TR	TR	1.4	TR	TR

Strains 1 CAU 1455^T, 2 *C. terreum* KCTC 19206^T, 3 *C. funkei* KCTC 39619^T, 4 *C. marinum* NBRC 110994^T, 5 *C. cellulans* KCTC 1771^T, 6 *C. aquatile* KCTC 39527^T. Data were from this study. Only those fatty acids amounting to > 1.0% in all strains are shown. *TR* trace amount (< 1.0%)

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Compliance with Ethical Standards

Conflict of interest The authors have declared that no competing interests exist.

Ethical statement The authors have declared that no ethical issues exist.

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