INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Sidhu *et al.*, *Int. J. Syst. Evol. Microbiol.* 2021;71:004664

DOI 10.1099/ijsem.0.004664

MICROBIOLOGY SOCIETY

Arenibacter amylolyticus sp. nov., an amylase-producing bacterium of the family *Flavobacteriaceae* isolated from marine water in India

Chandni Sidhu[1]†, Mohit Kumar Saini[1]‡, Naga Radha Srinivas Tanuku[2] and Anil Kumar Pinnaka[1,*]

Abstract

A novel Gram-stain-negative, curved rod-shaped, 0.5–0.7 µm wide and 3.0–10.0 µm long, non-motile bacterium, designated strain AK53^T, was isolated from a 5 m depth water sample collected from the Bay of Bengal, Visakhapatnam, India. Colonies on marine agar were circular, small, dark orange, shiny, smooth, translucent, flat, with an entire margin. The major fatty acids included iso-$C_{15:0}$, iso-$C_{15:0}$ 3OH, anteiso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3OH and summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$ and/or iso-$C_{15:0}$-2OH). Polar lipids included phosphatidylethanolamine and five unidentified lipids. The DNA G+C content of the strain AK53^T was found to be 40.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain AK53^T was closely related to *Arenibacter latericius* KMM 426^T and *Arenibacter certesii* KMM3941^T (pair-wise sequence similarity of 99.17 and 98.89 %, respectively), forming a distinct branch within the genus *Arenibacter* and clustering with *A. latericius*. Strain AK53^T shared average nucleotide identity (ANIb, based on BLAST) of 78.07 and 77.44% with *A. latericius* JCM 13508^T and *A. certesii* JCM 13507^T, respectively. Based on the observed phenotypic, chemotaxonomic characteristics and phylogenetic analysis, strain AK53^T is described in this study as representing a novel species in the genus *Arenibacter*, for which the name *Arenibacter amylolyticus* sp. nov. is proposed. The type strain of *Arenibacter amylolyticus* is AK53^T (=MTCC 12004^T= JCM 19206^T=KCTC 62553^T).

The genus *Arenibacter* is a member of the family *Flavobacteriaceae*, order *Flavobacteriales*, class *Flavobacteria*, phylum *Bacteroidetes*, and was established by Ivanova *et al.* [1]. Members of the genus *Arenibacter* are rod-shaped with slightly irregular sides and rounded ends, non-motile or motile by gliding. Colonies are regular, round, shiny, convex and produce non-diffusible, dark-orange carotenoid pigments. They are strictly aerobic, with chemoorganotrophic metabolism. Positive for oxidase, catalase and alkaline phosphatase. Gelatin, urea, DNA and Tweens 20 and 40 can be hydrolysed but not agar, casein, starch, alginic acids, cellulose, chitin or Tween 80. Some species require seawater or Na^+ for growth. Indole and H_2S are not produced. Major cellular fatty acids are iso-$C_{15:1}$ G, iso-$C_{15:0}$, $C_{15:0}$, iso-$C_{17:0}$ 3-OH and summed feature 3 (comprising $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c* and/or iso-$C_{15:0}$ 2-OH), the major polar lipid is a phosphatidylethanolamine and DNA G+C content is 37–41 mol%. The type species is *Arenibacter latericius* [1]. The genus *Arenibacter* presently comprises ten validly described species (https://lpsn.dsmz.de/genus/arenibacter) and species have been isolated from different substrates and geographic locations such as green alga *Ulva fenestrata* [2, 3], sea urchin *Strongylocentrotus intermedius* [4], surface sea water [5], marine tidal flat sediment [6, 7], marine bottom sediment [8, 9] and marine sandy sediment [1, 10]. In the present study, we focused on the characterization and classification of strain AK53^T [11], which was isolated from a 5 m depth marine water sample collected from Visakhapatnam, India, and based on our polyphasic taxonomic results assign the novel strain to the genus *Arenibacter*.

Strain AK53^T was isolated from a marine water sample collected at 5 m depth in Visakhapatnam, Andhra Pradesh,

Author affiliations: [1]MTCC-Microbial Type Culture Collection & Gene Bank, CSIR-Institute of Microbial Technology, Chandigarh 160036, India; [2]CSIR-National Institute of Oceanography, Regional Centre, 176, Lawsons Bay Colony, Visakhapatnam 530017, India.
***Correspondence:** Anil Kumar Pinnaka, apinnaka@imtech.res.in
Keywords: *Arenibacter amylolyticus*; 16S rRNA gene-based phylogeny; chemotaxonomy.
Abbreviations: ANI, average nucleotide identity; FAMEs, fatty acid methyl esters; L, unidentified polar lipid; MB, marine broth; MIDI, Microbial Identification System; PE, phosphatidylethanolamine; ZMA, Zobell's marine agar.
†Present address: Department of Molecular Ecology, Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, D-28359, Bremen, Germany
‡Present address: Department of Biological Sciences, Tokyo Metropolitan University, 1-1 Minami-Osawa, Hachioji, Tokyo 1920397, Japan.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AK53^T is HG529986. The GenBank/EMBL/DDBJ accession number for the whole genome of strain AK53^T is MTAZ00000000.
One supplementary figure and one supplementary table are available with the online version of this article.

India (17° 42′ 17.79″ N 83° 19′ 09.82″ E). For isolation of strain AK53^T, 1 ml water sample was serially diluted in 2% saline water, and 100 µl of which was plated on Zobell's marine agar (ZMA; HiMedia) and incubated for 10 days at 30 °C. Among the different colony morphotypes that appeared, a dark orange-coloured colony was selected and further characterized. Strain AK53^T was preserved with 10% glycerol in marine broth (MB; HiMedia) at −80 °C for long-term storage.

The type strains of *Arenibacter latericius* JCM 13508^T and *Arenibacter certesii* JCM 13507^T were also cultivated under the same conditions as strain AK53^T for comparative studies.

Isolation of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were performed as described in Surendra *et al.* [12]. The 16S rRNA gene sequence obtained was 1461 bp long and subjected to an EzBioCloud search (http://eztaxon-e.ezbiocloud.net/) [13] to find the nearest taxa. The 16S rRNA gene sequences of species of the genus *Arenibacter* and related species from different genera *Maribacter*, *Pibocella*, *Eudoraea*, *Kriegella* and *Zobellia* were downloaded from NCBI (www.ncbi.nlm.nih.gov) and aligned using the ClustalW tool of MEGA version 7 [14]. The neighbour-joining method was used to reconstruct the phylogenetic tree using MEGA. There was a total of 29 sequences and 1415 positions in the final dataset. Bootstrap analysis of 1000 replicates was performed to infer the robustness of the phylogenetic tree and the evolutionary distances were calculated using Kimura's two-parameter model [15]. The evolutionary history was inferred by using the maximum composite likelihood method based on the Tamura–Nei model [16]. A phylogenomic tree was reconstructed using ANVIO version 6.2 (available from http://merenlab.org/software/anvio/) using the workflow outlined by Eren *et al.* [17]. Briefly, contig databases were generated for all genomes using 'anvi-gen-contigs-database' program and the 'anvi-run-hmms' program was used to run default HMM profiles on the resulting contig databases. All common genes present in HMM source 'Bacteria_71' which contains 71 bacterial single-copy genes [18] were taken, and concatenated amino acid sequences of all these genes were used to create a phylogenomic tree using the FastTree program [19].

The cell morphology was studied by light microscopy (Zeiss; at ×1000). The cells were studied after staining with 1% phosphotungstic acid and drying on a carbon-coated copper grid and viewed under a transmission electron microscope (JEM 2100, JEOL). Cell motility was investigated as described by Bernardet *et al.* [20] and Gram staining was done using a Gram staining kit (HiMedia) according to the manufacturer's instructions. The colony morphology of strain AK53^T was examined following growth on ZMA for 48 h at 30 °C. Growth at 4, 10, 15, 20, 25, 30, 37, 45, 55 and 65 °C was ascertained using MB and salt tolerance [0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12% (w/v) NaCl] was ascertained using nutrient broth containing peptone (5 g l^{-1}) and beef extract (3 g l^{-1}) [21]. Growth of strain AK53^T at pH 4, 5, 6, 7, 8, 9, 10, 11 and 12 was assessed on MB buffered either with acetate or citrate buffer (pH 4–6), [100 mM NaH_2PO_4/Na_2HPO_4] buffer (pH 7–8), 100 mM $NaHCO_3/Na_2CO_3$ buffer (pH 9–10) or 100 mM Na_2CO_3/NaOH buffer (pH 11–12) [21]. Different biochemical tests listed in the description of species and in Table 1 were carried out for both strains under identical conditions using cultures grown at 37 °C on marine agar (MA) medium as described by Lányí [22]. Substrate hydrolysis (aesculin, casein, gelatin, starch and Tweens) and biochemical characteristics (catalase and oxidase activity, production of H_2S and reduction of nitrate, Voges–Proskauer and methyl red test, oxidation–fermentation tests, oxidation of tetramethyl-*p*-phenylenediamine dihydrochloride) were determined as previously described [22, 23]. The utilization of different carbohydrate sources was determined by analysing acid production by the isolate using bromocresol purple as an acid-base indicator [24]. ONPG discs were used to determine the *β*-galactosidase activity (HiMedia). Biochemical characterization of the different enzyme activities of strain AK53^T was also performed using the Vitek 2 GN system (bioMérieux) according to the manufacturer's protocol. The inoculum for Vitek analysis was prepared using sterile 2.0% (w/v) NaCl and incubated at 30 °C. Antibiotic sensitivity was determined using commercially available antibiotic discs (HiMedia) as described by Baek *et al.* [25]. Standardization of the physiological age of strains AK53^T, *A. latericius* JCM 13508^T and *A. certesii* JCM 13507^T was done based on the protocol [26] given by Sherlock Microbial Identification System (MIDI). For cellular fatty acid analysis, all strains were grown on ZMA plates at 30 °C for 2 days and were of the same physiological age (at the logarithmic phase of growth). Cellular fatty acid methyl esters (FAMEs) were obtained from cells by saponification, methylation and extraction following the MIDI protocol. Cellular FAMEs were separated by GC (6890, Agilent) and analysed using the Sherlock Microbial Identification System (MIDI-6890, Agilent; with database TSBA6) according to the protocol described by Sherlock Microbial Identification System. Extraction of polar lipids was performed as described in Bligh and Dyer [27] and analysed using two-dimensional TLC followed by spraying with suitable detection reagents [28].

Strain AK53^T was grown in MB and DNA was extracted using ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research). Whole genome sequencing was performed using Illumina Miseq 150×2 PE chemistry by the Interpret Omics centre for microarray and NGS (Banglore, India). *De novo* genome assembly was performed using CLC genomics workbench version 7.5.1 (Qiagen). rRNA and tRNA searches were performed using the RNAmmer version 1.2 [29] and ARAGORN version 1.2.36 servers [30], respectively. The completeness of the AK53^T genome was evaluated using checkM [31]. The G+C content of strains was determined by using whole genome sequences. The dbCAN2 server (http://bcb.unl.edu/dbCAN2/) was used to extract starch utilization operons from the genome of strain AK53^T [32].

For phylogenetic assessment of strain AK53^T, 1461 bp of 16S rRNA gene sequence was compared with the database of type strains with validly published names

Table 1. List of distinguishing features of strain AK53^T and closely related type strains of the genus *Arenibacter*

Strains: 1, AK53^T; 2, *Arenibacter latericius* JCM 13508^T; 3, *Arenibacter certesii* JCM 13507^T. +, Positive; –, negative; w, weak; S, susceptible; R, resistant.

Characteristics	1	2	3
Cell shape	Curved rods	Rod	Rod
Cell size (μm)	0.5–0.7×3.0–10.0	0.4–0.7×3.0–5.0	0.4–0.6×3.0–5.0
Temperature range for growth (°C)	25–37	10–37	4–37
Salinity range for growth (%, w/v)	2–8	1–8	1–10
pH range for growth (optimum)	6–9 (7)	6–10 (7–8)	6–11 (7)
Starch hydrolysis	+	–	–
Nitrate reduction to nitrite	–	+	–
Tween 40/60 hydrolysis	+/–	+/+	–/–
Carbohydrate utilization:			
Maltose	+	–	+
Mannose	+	w	–
Melibiose	–	–	+
Raffinose	–	–	+
Sucrose	–	+	–
Fructose	+	–	+
Xylose	w	–	–
Rhammnose	–	–	+
Galactose	–	–	+
Lactose	+	w	–
Vitek characterization:			
γ-Glutamyl-transferase	+	–	–
β-Xylosidase	+	–	–
L-Proline arylamidase	+	–	–
β-*N*-Acetylgalactosaminidase	+	+	–
β-Galactosidase	+	+	–
Glycine arylamidase	+	+	–
Ellman reaction	w	–	–
Streptomycin susceptibility (25 (μg/disc)	S	S	R
G+C content (mol%)	40.8	36.8	37.7
Habitat*	Marine water from 5 m depth, Bay of Bengal	Sediment from 20 m depth, South China Sea	Green alga, *Ulva fenestrata*, Sea of Japan

*Data for type strains taken from Ivanova *et al.* [1] and Nedashkovskaya *et al.* [2].

(http://eztaxon-e.ezbiocloud.net/), which revealed its affiliation to the genus *Arenibacter* of the family *Flavobacteriaceae*. Based on pair-wise sequence similarity, strain AK53^T was close to *A. latericius* KMM 426^T and *A. certesii* KMM3941^T with values of 99.17 and 98.89%, respectively. With other members of the genus *Arenibacter*, pair-wise sequence similarity was between 94.35–96.59%. Based on the neighbour-joining tree, strain AK53^T formed a distinct branch within the genus *Arenibacter* and clustered with *A. latericius* KMM 426^T and together clustered with *A. certesii* KMM3941^T (Fig. 1a). Similar clustering was observed in a phylogenomic tree reconstructed using 71 bacterial single-copy genes (Fig. 1b).

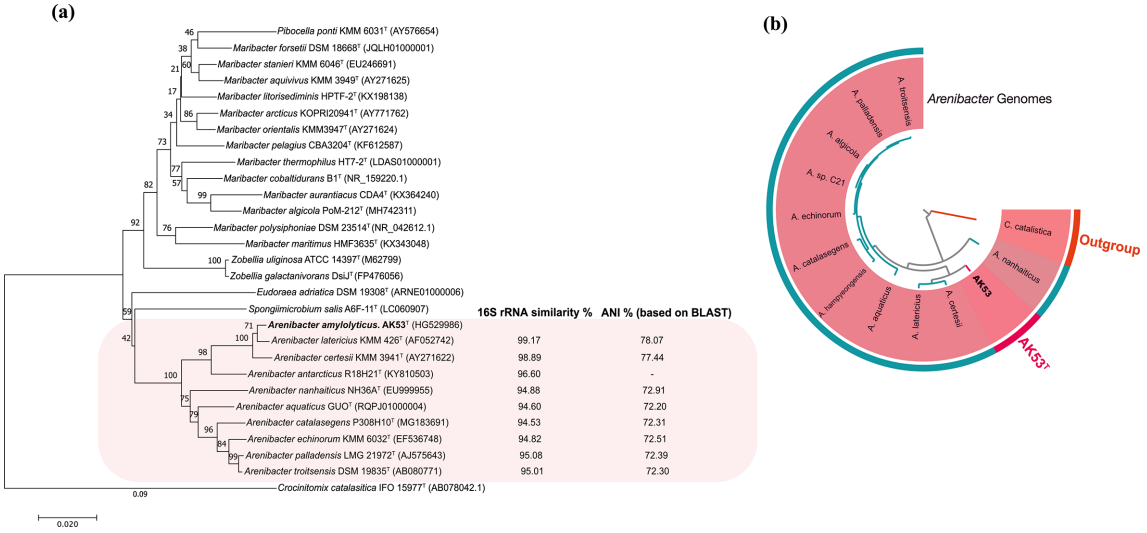

Fig. 1. (a). Neighbour-joining tree showing phylogenetic relationships of strain AK53^T with representative members of the family *Flavobacteriaceae* based on the 16S rRNA sequence dataset. Bootstrap support values are shown at the nodes. *Crocinitomix catalasitica* IFO 15977^T (AB078042) was used as an outgroup. Scale bar represents 0.01 substitutions per nucleotide positions. (b). Phylogenomic tree of the genus *Arenibacter* showing clustering of species based on bacterial single-copy genes.

Cells of strain AK53^T were Gram-negative, curved rod-shaped, 0.5–0.7 µm wide and 3.0–10.0 µm long, aerobic and non-motile (Fig. 2). Colonies on MA were circular, dark orange, shiny, smooth, translucent, small, flat, with an entire margin. The temperature growth range for AK53^T was 25–37 °C, with optimum growth at 30 °C. Growth was observed at 2–8 % NaCl (w/v) with optimum growth at 2% NaCl and at pH 6–9, with optimum growth at pH 7. The genomic DNA G+C content of strain AK53^T was 40.8 mol%. A list of distinguishing features between strain AK53^T and its reference strains is provided in Table 1.

The major fatty acids of strain AK53^T included iso-$C_{15:0}$, iso-$C_{15:0}$ 3OH, anteiso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3OH and summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$ and/or iso-$C_{15:0}$-2OH) (Table 2). *A. latericius* JCM 13508^T and *A. certesii* JCM 13507^T had similar major fatty acid compositions, but they had differences with respect to the presence of a few fatty acids, the absence of few fatty acids and variations in concentrations (Tables 1 and 2). The polar lipids of strain AK53^T included phosphatidylethanolamine (PE) and five unidentified lipids (L1–L5) (Fig. 3a). However, the reference strains *A. latericius* JCM 13508^T and *A. certesii* JCM 13507^T had similar compositions (PE and L1–L5). *A. latericius* JCM 13508^T had one unidentified lipid (L6) extra (Fig. 3a, b). The DNA G+C content of strain AK53^T was found to be 40.8 mol%.

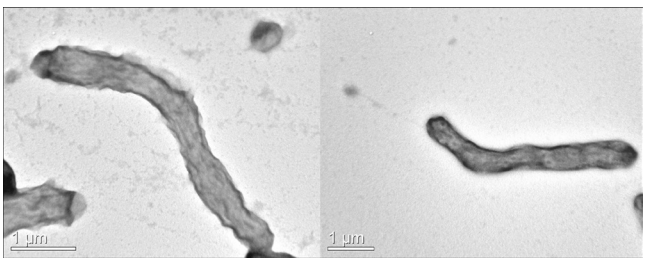

Fig. 2. Electron microscopy image of negatively stained cells of strain AK53^T. Bar, 1.0 µm.

JSpeciesWS was used to analyse average nucleotide identity based on BLAST (ANIb) [33]. The results suggest that strain AK53^T shares 78.07 and 77.44 % ANIb with *A. latericius* JCM 13508^T and *A. certesii* JCM 13507^T respectively (Fig. 1a). As ANI values above 95% are indicative of the same species [34], our strain AK53^T belongs to the genus *Arenibacter* but is different from other species in the genus.

The *de novo* assembly of the whole genome of strain AK53^T contained 101 contigs consisting of 3985366 bp, with a G+C content of 40.8 mol%. NCBI annotation suggested a total of 3208 protein-coding open reading frames. All three types of rRNAs (16S rRNA, 5S rRNA, 23S rRNA) were identified by the RNAmmer 1.2 server and ARAGORN version 1.2.36 predicted a total of 48 tRNAs in the genome. CheckM results showed that strain AK53^T had a completeness of 99.67%. dbCAN2 server results showed the presence of three starch-utilization operons. Each operon has at least a single copy of α-amylase (EC 3.2.1.1) and a corresponding sugar transporter suggesting active degradation of starch in stain AK53^T (Fig. S1, available in the online version of this article).

A general comparison of the genomic features of strain AK53^T, *A. latericius* JCM 13508^T and *A. certesii* JCM 13507^T is given in Table S1.

Table 2. Comparison of the fatty acid compositions of strain AK53^T and those of closely related type strains of the genus *Arenibacter*

Strains: 1, AK53^T; 2, *Arenibacter latericius* JCM 13508^T; 3, *Arenibacter certesii* JCM 13507^T. All data from the present study. Results are presented as a percentage of the total fatty acids. Fatty acids amounting to 5% or more of the total fatty acids are in bold. ND, Not detected. TR, traces (<1%). Values of less than 1% for all strains are not shown.

Fatty acid	1	2	3
iso-$C_{13:0}$	TR	TR	1.0
iso-$C_{14:0}$	ND	TR	1.7
$C_{15:0}$ 2OH	1.0	2.6	TR
$C_{15:0}$ 3OH	2.5	ND	ND
iso-$C_{15:0}$	**17.7**	**11.7**	**12.4**
iso-$C_{15:0}$ 3OH	**5.6**	4.3	**5.3**
anteiso-$C_{15:0}$	**5.4**	**8.4**	3.3
iso-$C_{15:1}$ G	**9.0**	**5.4**	**11.0**
$C_{15:1}$ *ω6c*	4.6	4.3	3.8
$C_{16:0}$	3.1	**5.0**	3.5
$C_{16:0}$ 3OH	1.8	2.9	1.5
iso-$C_{16:0}$	1.0	2.8	TR
iso-$C_{16:0}$ 3OH	1.9	1.9	3.3
anteiso-$C_{16:0}$	1.0	TR	ND
iso-$C_{16:1}$ h	TR	1.6	TR
$C_{17:0}$ 10-methyl	ND	1.6	ND
$C_{17:0}$ 2OH	1.6	3.1	1.5
$C_{17:0}$ 3OH	1.3	3.9	1.3
iso-$C_{17:0}$ 3OH	**15.9**	**10.3**	**17.9**
anteiso-$C_{17:0}$	TR	1.0	TR
$C_{17:1}$ *ω6c*	1.3	2.6	2.4
$C_{17:1}$ *ω8c*	1.0	1.1	1.7
$C_{18:3}$ *ω*6,9,12*c*	1.1	1.1	0.3
$C_{19:0}$	ND	1.0	ND
iso-$C_{19:0}$	TR	1.2	TR
$C_{20:1}$ *ω7c*	ND	1.8	TR
Summed feature 3*	**12.4**	**9.0**	**14.6**
Summed feature 7*	TR	1.2	ND
Summed feature 9*	2.8	2.5	**5.0**

*Summed features are groups of two or three fatty acids that could not be separated by GC with the MIDI system. Summed feature 3 comprised $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c* and/or iso-$C_{15:0}$ 2-OH; summed feature 7 comprised $C_{19:1}$ *ω7c* and/or $C_{19:1}$ *ω6c* and/or $C_{19:0}$ cyclo *ω*10c; summed feature 9 comprised $C_{16:0}$ 10-methyl and/or iso-$C_{17:1}$ *ω*9c.

Based on the phylogenetic analysis, a characteristics comparison was made between strain AK53^T and relative type strains such as *A. latericius* JCM 13508^T and *A. certesii* JCM 13507^T of the family *Flavobacteriaceae* (Table 1) and a number of differences were observed. Strain AK53^T and the reference strains exhibited similar characteristics with respect to Gram-stain, cell shape, colour of cell suspension, H_2S and indole production, oxidase, catalase and several enzymatic activities, carbon substrate utilization and sensitivity to different antibiotics. However, strain AK53^T differed from the other type strains

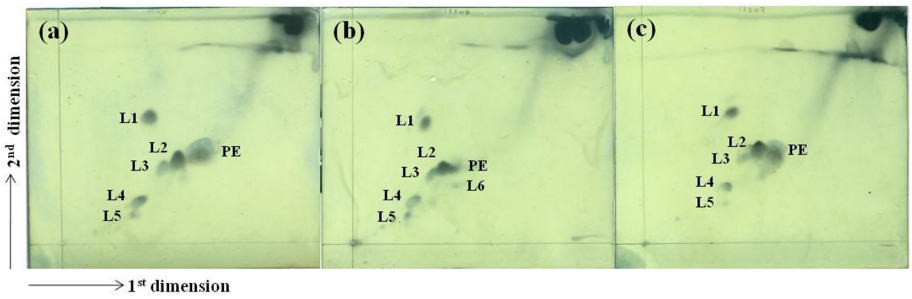

Fig. 3. Two-dimensional thin-layer chromatogram of the total polar lipids of the strain AK53^T (a), *Arenibacter latericius* JCM 13508^T (b), *Arenibacter certesii* JCM 13507^T (c), after spraying the plates with molybdatophosphoric acid. Abbreviations: PE, phosphatidylethanolamine; L, unidentified polar lipid. The spots identified as phospho-, amino- or glycolipids by spraying molybdenum blue, ninhydrin and α-naphthol reagents, respectively.

in several characteristics such as cell size, temperature growth range, salinity growth range, pH growth range and optimum, hydrolysis of starch, Tween 40/60, utilization of several carbon substrates, γ-glutamyl-transferase, *β*-xylosidase, L-proline arylamidase, *β*-*N*-acetyl galactosaminidase, *β*-galactosidase and glycine arylamidase activities, Ellman reaction, sensitivity to streptomycin (25), major fatty acids, polar lipids and G+C content (Table 1). Based on the observed phenotypic, chemotaxonomic characteristics and phylogenetic analyses, strain AK53^T is sufficiently different from the existing species in the genus *Arenibacter* of the family *Flavobacteriaceae*, to propose the name *Arenibacter amylolyticus* sp. nov.

DESCRIPTION OF *ARENIBACTER AMYLOLYTICUS* SP. NOV.

Arenibacter amylolyticus (a.my.lo.ly'ti.cus Gr. n. *amylon*, starch; N.L. masc. adj. *lyticus*, able to dissolve; from Gr. masc. adj. *lytikos*, dissolving; N.L. masc. adj. *amylolyticus*, starch-dissolving).

The major characteristics of strain AK53^T are similar to the description provided for the genus. Cells are Gram-stain-negative, aerobic, non-motile, curved rods, 0.5–0.7 µm wide and 3.0–10.0 µm long. Colonies on MA are circular, dark orange, shiny, smooth, translucent, small, flat, with an entire margin. Growth occurs at 25–37 °C (optimum, 30 °C), 2–8% (w/v) NaCl (optimum, 2% NaCl w/v) and at pH 6–9 (optimum, pH 7). Oxidase- and catalase-positive. Positive for hydrolysis of starch, gelatin, aesculin and Tween 40 and utilization of maltose, mannose, fructose, lactose and trehalose; weakly positive for xylose. Negative for methyl red and Voges–Proskauer's reactions, indole production, nitrate reduction, hydrolysis of casein and Tweens 20/60/80, and utilization of melibiose, raffinose, dextrose, sucrose, rhamnose, salicin, cellobiose, galactose, dulcitol, sorbitol, inulin, inositol, adonitol and mannitol. Vitek GN test positive for Ala-Phe-Pro-arylamidase, *β*-galactosidase, *β*-*N*-acetylglucosaminidase, glutamyl arylamidase, γ-glutamyl-transferase, *β*-glucosidase, *β*-xylosidase, L-proline arylamidase, tyrosine arylamidase, urease, α-glucosidase, *β*-*N*-acetyl galactosaminidase, *β*-galactosidase, phosphatase, glycine arylamidase, glu-gly-arg-arylamidase activities, weak for the Ellman reaction, and negative for L-pyrrolidinyl-arylamidase, *β*-alanine arylamidase, lipase, lysine and ornithine decarboxylase and *β*-glucuronidase activities; adonitol, L-arabitol, cellobiose, D-glucose, maltose, D-mannitol, D-mannose, palatinose, D-sorbitol, sucrose, D-tagatose, trehalose, citrate (sodium), malonate, 5-keto-D-gluconate and courmarate utilization; H_2S production, fermentation of glucose, L-lactate and succinate alkalinization, L-histidine L-lactate and L-malate assimilation, 0/129 resistance. Resistant to gentamicin, neomycin and kanamycin and sensitive to ampicillin, rifampicin, lincomycin and streptomycin. The major fatty acids include iso-$C_{15:0}$, iso-$C_{15:0}$ 3OH, anteiso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3OH and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c* and/or iso-$C_{15:0}$-2OH). Polar lipids include phosphatidylethanolamine and five unidentified lipids. The DNA G+C content of strain AK53^T is 40.8 mol%.

The type strain, AK53^T (=JCM 19206^T=KCTC 62553^T=MTCC 12004^T), was isolated from a water sample collected at 5 m depth in the Bay of Bengal, Visakhapatnam, Andhra Pradesh, India.

Funding information
The work is supported by funds received from CSIR project BSC402.

Acknowledgements
The authors thank the Directors, Institute of Microbial Technology and National Institute of Oceanography. We thank the Council of Scientific and Industrial Research (CSIR) and the Department of Biotechnology, Government of India for financial assistance. T.N.R.S. is thankful to project OLP1710 for providing facilities and funding, respectively. We also thank Mr. Deepak Bhatt for technical aid in sequencing the 16S rRNA gene. C.S. thanks CSIR-UGC for the research fellowship.

Author contributions
C.S. T.N.R. and A.K. conceived and designed the research. T.N.R. performed sample collection. C.S. and M.K. performed the experiments. C.S., T.N.R. and A.K. analysed the data. T.N.R. and C.S. wrote the original manuscript. C.S., A.K. and M.K. reviewed the manuscript. All authors contributed to the manuscript and approved the submitted version.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Ivanova EP, Nedashkovskaya OI, Chun J, Lysenko AM, Frolova GM *et al*. *Arenibacter* gen. nov., new genus of the family *Flavobacteriaceae* and description of a new species, *Arenibacter latericius* sp. nov. *Int J Syst Evol Microbiol* 2001;51:1987–1995.
2. Nedashkovskaya OI, Kim SB, Han SK, Lysenko AM, Mikhailov VV *et al*. *Arenibacter certesii* sp. nov., a novel marine bacterium isolated from the green alga *Ulva fenestrata*. *Int J Syst Evol Microbiol* 2004;54:1173–1176.
3. Nedashkovskaya OI, Vancanneyt M, Cleenwerck I, Snauwaert C, Kim SB *et al*. *Arenibacter palladensis* sp. nov., a novel marine bacterium isolated from the green alga *Ulva fenestrata*, and emended description of the genus *Arenibacter*. *Int J Syst Evol Microbiol* 2006;56:155–160.
4. Nedashkovskaya OI, Kim SB, Lysenko AM, Lee KH, Bae KS *et al*. *Arenibacter echinorum* sp. nov., isolated from the sea urchin *Strongylocentrotus intermedius*. *Int J Syst Evol Microbiol* 2007;57:2655–2659.
5. Guo J, Sun J, Xu Y, Fang L, Jiao N *et al*. *Arenibacter aquaticus* sp. nov., a marine bacterium isolated from surface sea water in the South China Sea. *Int J Syst Evol Microbiol* 2020;70:958–963.
6. Jeong SH, Jin HM, Kim JM, Jeon CO. *Arenibacter hampyeongensis* sp. nov., a marine bacterium isolated from a tidal flat. *Int J Syst Evol Microbiol* 2013;63:679–684.
7. Li A-Z, Lin L-Z, Zhang M-X, Lv Y, Zhu H-H. *Arenibacter catalasegens* sp. nov., isolated from marine surface sediment, and emended description of the genus *Arenibacter*. *Int J Syst Evol Microbiol* 2018;68:758–763.
8. Nedashkovskaya OI, Suzuki M, Vysotskii MV, Mikhailov VV. *Arenibacter troitsensis* sp. nov., isolated from marine bottom sediment. *Int J Syst Evol Microbiol* 2003;53:1287–1290.
9. Li A-Z, Lin L-Z, Zhang M-X, Zhu H-H. *Arenibacter antarcticus* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* 2017;67:4601–4605.
10. Sun F, Wang B, Du Y, Liu X, Lai Q *et al*. *Arenibacter nanhaiticus* sp. nov., isolated from marine sediment of the South China Sea. *Int J Syst Evol Microbiol* 2010;60:78–83.
11. Vandamme P, Pot B, Gillis M, de Vos P, Kersters K *et al*. Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol Rev* 1996;60:407–438.
12. Surendra V, Bhawana P, Suresh K, Srinivas TNR, Anil Kumar P. *Imtechella halotolerans* gen. nov., sp. nov., a member of the family *Flavobacteriaceae* isolated from estuarine water. *Int J Syst Evol Microbiol* 2012;62:2624–2630.
13. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y *et al*. Introducing EzBioCloud: a taxonomically United database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
14. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
15. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
16. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 1993;10:512–526.
17. Eren AM, Esen Özcan C, Quince C, Vineis JH, Morrison HG *et al*. Anvi'o: an advanced analysis and visualization platform for 'omics data. *PeerJ* 2015;3:e1319.
18. Lee MD. GToTree: a user-friendly workflow for phylogenomics. *Bioinformatics* 2019;35:4162–4164.
19. Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Mol Biol Evol* 2009;26:1641–1650.
20. Bernardet JF, Nakagawa Y, Holmes B. Subcommittee on the taxonomy of *Flavobacterium* and *Cytophaga*-like bacteria of the International Committee on Systematics of Prokaryotes. Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* 2002;52:1049–1070.
21. Srinivas TNR, Aditya S, Bhumika V, Kumar PA, Bhumika V. *Lunatimonas lonarensis* gen. nov., sp. nov., a haloalkaline bacterium of the family *Cyclobacteriaceae* with nitrate reducing activity. *Syst Appl Microbiol* 2014;37:10–16.
22. Lányí B. Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* 1987;19:1–67.
23. Cowan ST, Steel KJ. *Manual for the Identification of Medical Bacteria*, 149. New York: Cambridge University Press; 1965. p. 852.
24. Gordon RE, Barnett DA, Handerhan JE, Pang CH-N. *Nocardia coeliaca*, *Nocardia autotrophica*, and the Nocardin strain. *Int J Syst Bacteriol* 1974;24:54–63.
25. Baek S-H, Cui Y, Kim S-C, Cui C-H, Yin C *et al*. *Tumebacillus ginsengisoli* sp. nov., isolated from soil of a ginseng field. *Int J Syst Evol Microbiol* 2011;61:1715–1719.
26. Sasser M. Identification of bacteria through fatty acid analysis. In: Klement Z, Rudolph K, Sands Budapest DC (editors). *Methods in Phytobacteriology*. Hungry: Akademiai Kiado; 1990. pp. 199–204.
27. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911–917.
28. Komagata K, Suzuki K. Lipid and cell wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–207.
29. Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T *et al*. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 2007;35:3100–3108.
30. Laslett D, Canback B. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Res* 2004;32:11–16.
31. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;25:1043–1055.
32. Zhang H, Yohe T, Huang L, Entwistle S, Wu P *et al*. dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. *Nucleic Acids Res* 2018;46:W95–W101.
33. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J, Glo FO. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 2016;32:929–931.
34. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P *et al*. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.