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***Caldimonas meghalayensis* sp. nov., a novel thermophilic betaproteobacterium isolated from a hot spring of Meghalaya in northeast India**

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## Abstract

While studying the microbial diversity of hot spring of North-east India we isolated a strain AK31<sup>T</sup> from the Jakrem hot spring of Meghalaya. The strain formed light yellow colony on nutrient agar and was Gram negative, non spore-forming rods, motile with single polar flagellum. The strain was positive for oxidase and catalase and hydrolysed starch and weakly urea. The predominant cellular fatty acids were C<sub>16:0</sub> (34.8%), C<sub>17:0</sub> cyclo (27.1%), C<sub>16:1</sub> ω7c and/or iso-C<sub>15:0</sub> 2OH (summed feature 3) (9.6%), C<sub>10:0</sub> 3OH (8.0%), C<sub>12:0</sub> (5.8%), C<sub>14:0</sub> (5.3%) and C<sub>18:1</sub> ω7c (5.3%). Strain AK31<sup>T</sup> contained ubiquinone-8 as the major respiratory quinone and diphosphatidylglycerol, phosphatidylethanolamine, three unidentified phospholipids and one unidentified glycolipid as the polar lipids. The G + C content of the DNA of the strain AK31<sup>T</sup> was 66.7 mol%. The 16S rRNA gene sequence analysis indicated that strain AK31<sup>T</sup> was member of the genus *Caldimonas* and closely related to *C. manganoxidans* JCM 10698<sup>T</sup> and *C. taiwanensis* On1<sup>T</sup> with 96.9% similarity and with *Aquicola tertiaricarbonis* L10<sup>T</sup> and *Azohydromonas australica* IAM 12664<sup>T</sup> with 96.5% and 96.4% similarity respectively. Phylogenetic analyses indicated that the strain AK31<sup>T</sup> clustered with *C. manganoxidans* JCM 10698<sup>T</sup> and *C. taiwanensis* On1<sup>T</sup> with a phylogenetic distance of 3.25%. Based on data from the current polyphasic study, strain AK31<sup>T</sup> is proposed as a novel species of the genus *Caldimonas*, for which the name *Caldimonas meghalayensis* sp. nov. is proposed. The type strain of *C. meghalayensis* is AK31<sup>T</sup> (= MTCC 11703<sup>T</sup> = JCM 18786<sup>T</sup>).

**Key words:** *Caldimonas meghalayensis* · North-East India · 16S rRNA gene based phylogeny · chemotaxonomy · *Betaproteobacteria*

## Introduction

The north-eastern region in India has rich microbial diversity with potential of bioprospecting as it is among the 12 mega biodiversity rich zones of the world forming a distinctive part of the Indo-Burma Hotspot (Myers 2000). We explored the bacteria from the hot spring of this region as the extreme environments are evident of rich diversity and for the bio-prospecting of heat loving bacteria which can provide thermostable novel enzymes. While studying the bacteria from Jakrem, a hot spring of Meghalaya we obtained a strain designated AK31<sup>T</sup> whose phylogenetic analysis based on 16S rRNA gene sequence showed that it is closely related to the genus *Caldimonas* of the family *Comamonadaceae*. The genus *Caldimonas* comprises Gram-negative, motile by single polar flagellum, rod-shaped, oxidase and catalase positive, thermo-alkali-tolerant, chemoorganoheterotrophic, aerobic bacteria with accumulated PHB granules as storage material and ubiquinone-8 as major respiratory quinone, C<sub>16:0</sub>, C<sub>16:1</sub> and C<sub>18:1</sub> as major cellular fatty acids and the DNA G + C content ranging between 65.9 and 66.2 mol% (Takeda et al. 2002; Chen et al. 2005). At the time of writing, the genus *Caldimonas* is represented by two validly published species, *C. manganoxidans* JCM 10698<sup>T</sup> and *C. taiwanensis* On1<sup>T</sup> which were isolated from the hot spring in Japan (Takeda et al. 2002) and the hot spring in Taiwan (Chen et al. 2005) respectively. *C. taiwanensis* On1<sup>T</sup> is a taxonomic group of *C. manganoxidans* with no distinguishable 16S rRNA gene sequence (the taxonomic group information is acquired from <http://eztaxon-e.ezbiocloud.net/>; Kim et al. 2012) but distinguished based on DNA-DNA hybridization and a number of phenotypic and chemotaxonomic characters (Chen et al. 2005). In the present study we focused on the characterization and classification of the strain AK31<sup>T</sup>, which was isolated from hot spring, using polyphasic approach (Vandamme et al. 1996). The strain was affiliated to the genus *Caldimonas* and is proposed as a novel species, *C. meghalayensis* sp. nov.

## Materials and Methods

### Isolation and preservation

Strain AK31<sup>T</sup> was isolated from a water sample collected from Jakrem hot spring in Meghalaya of North-east India (GPS positioning 25° 24.451'N 91° 32.415'E). The sample temperature observed was 50 °C and the pH was 9. The water of the thermal spring is reported to be high in sulphur and calcium with minute concentration of phosphate and nitrate (Siangbood and Ramanujam 2011). For isolation serial tenfold dilution of soil samples were made with 0.85% saline water to plate in triplicate on R2A agar media (pH 7.0) (HIMEDIA, India) and incubated at 50 °C for 72 h. Out of the different morphotypes obtained one light yellow colony was selected and characterized in the present study. The purity of the culture was confirmed by repeated streaking on R2A agar media and by microscopic observation. Strain AK31<sup>T</sup> was maintained on R2A slants at 4°C and was preserved using 15 % glycerol at -80 °C.

### Type strains used for comparative study

*Caldimonas manganoxidans* JCM 10698<sup>T</sup> was collected from Japan collection of microorganism (JCM), Japan and *Caldimonas taiwanensis* LMG 22827<sup>T</sup> was collected from Belgian co-ordinated collections of micro-organisms (BCCM), Belgium. Further characterization of strain AK31<sup>T</sup> was carried simultaneously with the procured type strains.

### Morphological characteristics

The isolated pure colony was checked for cell morphology and motility by using phase contrast microscopy (Olympus, USA); the cells were harvested by centrifugation and negatively stained with 2% (w/v) uranyl acetate then subjected to transmission electron microscopy (Jeol JEM 2100) at operating voltage of 200 kV to visualize the ultra structure of bacterium.

### Physiological and biochemical characteristics

Physiological and biochemical characteristics were determined as previously described in Anil Kumar et al. (2010) with a variation that incubation of the bacteria were performed at 50°C. Biochemical and enzymatic characterization were also performed using Vitek 2 GN kits (BioMérieux, France) with incubation at 50 °C, according to the manufacturer's protocol. Determinations of sensitivity to 24 different antibiotics were made using the disc diffusion method with commercially available discs (HIMEDIA) using previously described methods (Lányi 1987; Smibert and Krieg 1994).

### Chemotaxonomic analysis

Strain AK31<sup>T</sup>, *C. manganoxidans* JCM 10698<sup>T</sup> and *C. taiwanensis* LMG 22827<sup>T</sup> were grown to prepare fatty acid methyl esters (classical methods) and were analysed using Sherlock Microbial Identification System (MIDI-6890 with database TSBA6) by the protocol described by the manufacturer. Extraction and analysis of polar lipids were performed as described by Komagata and Suzuki (1987). Menaquinones were analyzed by HPLC (Groth et al. 1997) after performing extraction as described by Collins et al. (1977). Presence of PHB granules was checked by Nile blue A staining method as described by Ostle and Holt (1982).

### Determination of DNA G + C content

For determination of G+C content, genomic DNA of the strain AK31<sup>T</sup> was extracted following the protocol of Marmur (1961). Melting point (*T<sub>m</sub>*) curves (Sly et al. 1986) with Lambda 2 UV-Vis spectrophotometer (Perkin Elmer) equipped with the Templab 2.0 software package (Perkin Elmer) were used for G + C content determination. *Escherichia coli* strain DH5- $\alpha$  DNA was used as a standard.

### DNA–DNA hybridizations

Genomic DNA of strain AK31<sup>T</sup>, JCM10698<sup>T</sup> and LMG 22827<sup>T</sup> were extracted using bacterial DNA isolation kit (Zymo Research, USA). Total DNA from these strains were labelled with tritium-

labelled nucleotide using the Megaprime DNA labelling system (Amersham, UK). DNA-DNA hybridization experiments were performed at  $T_m - 20^\circ\text{C}$  for proper reassociation with prehybridization buffer (0.5M phosphate buffer with 7% SDS, pH 7.2) as described by Tourova and Antonov (1987). Hybridized DNA was detected densitometrically using labelled DNA as substrate with STARION next generation multi-purpose image scanner FLA 9000 (Fujifilm, Japan). The experiment was performed in duplicates and the DNA relatedness values are expressed as means of these two values.

#### Amplification of 16S rRNA gene sequence and phylogenetic analysis

A bacterial DNA isolation kit (Zymo Research, USA) was used to isolate DNA for 16S rRNA gene amplification. 16S rRNA gene sequences were amplified by PCR using two general bacterial 16S rRNA gene primers. PCR mixtures (25  $\mu\text{l}$ ) contained approximately 30 ng of DNA, 2  $\mu\text{M}$  forward primer (27F), 2  $\mu\text{M}$  reverse primer (1492R), 1.5 mM of  $\text{MgCl}_2$  (Taq Buffer), deoxynucleoside triphosphates (250  $\mu\text{M}$  each of dATP, dCTP, dGTP and dTTP) and 0.6 U of Taq polymerase. DNA amplification was carried out in Gene AMP PCR system 9700 (Applied Biosystems, USA). Approximately 1500 nucleotides were amplified using PCR. Templates replaced with sterile water were used as negative controls. Amplified products were treated with ExoSap (Affymetrix, China) before bi-directional sequencing using the forward, reverse and internal primers corresponding to *Escherichia coli* positions 357F, 926F, 685R and 1100R by Genetic Analyzer ABI 3130XL (Applied Biosystems, California, USA) with Big Dye (3.1) terminator protocol (Kumar et al. 2013).

EzBioCloud (Kim et al. 2012) was used to determine the phylogenetic neighbours against the database of type strains with validly published prokaryotic names. Molecular Evolutionary Genetics Analysis software (MEGA version 5) was used for phylogenetic analyses (Tamura et al. 2011). The sequences of identified phylogenetic neighbours were aligned with the sequence of the strain AK31<sup>T</sup> using Clustal W inbuilt with MEGA 5. *Burkholderia pseudomallei* 1026b<sup>T</sup> was used as the out group organism. Phylogenetic trees were constructed using tree-making algorithms; the neighbor-joining

and maximum likelihood methods using the MEGA5 package (Tamura et al. 2011) and the resultant tree topologies were evaluated based on 1000 resamplings. The 16S rRNA gene sequence (1410 bp) was submitted to NCBI with the accession number HF562216.

## Results and Discussion

A yellow coloured thermo tolerant bacterium from Jakrem hot spring in Meghalaya of Northeast India was isolated on R2A agar medium at 50 °C. The strain AK31<sup>T</sup> grew aerobically on R2A as well as on other complex medium like nutrient agar (NA; HIMEDIA, India), trypticase soy agar (TSA; HIMEDIA, India) and Luria-Bertani agar (LBA; HIMEDIA, India) without supplement of any growth factor. Colonies were circular, smooth, light yellow, opaque and convex with entire margin on nutrient agar (NA; HIMEDIA, India) plates. Transmission electron microscopy revealed the cells were non spore-forming rods with width of 0.4-0.6 µm and length of 1.4-2.0 µm and with single polar flagellum and possess granules (Fig. 1). The granules produced were PHB granules as determined by Nile Blue A staining. The novel strain was unable to grow in anaerobic condition. Growth was observed at 25–55 °C with optimum growth at 45 °C, at 0–2% (w/v) NaCl (with optimum growth without NaCl), and at pH 6–10 (with optimum growth at pH 7.0). Other characteristics are presented in Table 1 and also listed in the species description.

The cellular fatty acid composition of strain AK31<sup>T</sup> showed a profile of 8 fatty acids with a dominance of (>5%) of C<sub>16:0</sub>, C<sub>17:0</sub> cyclo, C<sub>16:1</sub> ω7c and/or iso-C<sub>15:0</sub> 2OH (summed feature 3), C<sub>10:0</sub> 3OH, C<sub>12:0</sub>, C<sub>14:0</sub> and C<sub>18:1</sub> ω7c (Table 2). Saturated fatty acids constituted 82.4 to 92% of the total fatty acids. The fatty acid profiles of type strains *C. manganoxidans* JCM 10698<sup>T</sup> and *C. taiwanensis* On1<sup>T</sup> were determined in the present study providing similar condition of growth and extraction and were observed to be differing noticeably compared with strain AK31<sup>T</sup> (Table 2). The respiratory quinone present in strain AK31<sup>T</sup> was ubiquinone-8 (UQ-8) and the major polar lipids consisted of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), three unidentified phospholipids

(PL) and one unidentified glycolipid (GL) (Fig. 2a). The strain AK31<sup>T</sup> differentiated with the type strain *C. manganoxidans* JCM 10698<sup>T</sup> and *C. taiwanensis* LMG 22827<sup>T</sup> in the occurrence of unidentified phospholipids (PL) (Fig. 2; Table 1). The G + C content of DNA of the strain AK31<sup>T</sup> was 66.7 mol%. The strain AK31<sup>T</sup> differentiated with the next phylogenetic relatives of the genus *Schlegelella* based on the G+C content (Supplementary Table S1) but was found closer to the genus *Caldimonas*.

For phylogenetic assessment of strain AK31<sup>T</sup>, 1410 bp of 16S rRNA gene sequence was amplified and sequenced. The sequence was compared with the database of type strains with validly published prokaryotic names [Available online <http://eztaxon-e.ezbiocloud.net/> (Kim et al. 2012)] which revealed its closeness to *Burkholderiales* group of the *Betaproteobacteria*. Based on pair-wise sequence similarity strain AK31<sup>T</sup> was closest to *C. manganoxidans* JCM 10698<sup>T</sup> and *C. taiwanensis* LMG 22827<sup>T</sup> with 96.9% similarity and to *Aquincola tertiaricarbonis* L10<sup>T</sup> and *Azohydromonas australica* IAM 12664<sup>T</sup> with 96.5% and 96.4% similarity respectively. Phylogenetic tree based on maximum likelihood method showed the clustering of strain AK31<sup>T</sup> with *C. manganoxidans* JCM 10698<sup>T</sup> and *C. taiwanensis* On1<sup>T</sup> with a phylogenetic distance of 3.25% (96.75% similarity) (Fig. 3). In neighbour-joining phylogenetic tree strain AK31<sup>T</sup> clustered with members of the genera *Caldimonas* and together clustered with members of the genus *Schlegelella* (Supplementary Fig. S1).

Strain AK31<sup>T</sup> differed from the type strains with respect to composition of cellular fatty acids, cell size, temperature growth range, pH growth range, biochemical characteristics, fatty acid and polar lipids composition and G + C content (Tables 1 and 2, Supplementary Table S1). To differentiate the proposed novel strain with the nearest thermo tolerant bacteria, DNA–DNA hybridization experiment was performed between the strain AK31<sup>T</sup> and the closest phylogenetic neighbour viz., *C. manganoxidans* JCM 10698<sup>T</sup> and *C. taiwanensis* LMG 22827<sup>T</sup>. The average relatedness of the studied strain with *C. manganoxidans* JCM 10698<sup>T</sup> was 31.5% and with *C. taiwanensis* LMG 22827<sup>T</sup> was 56.5%. The novel strain has DNA G+C content (mol%) and major



fatty acids profile closer to the *Caldimonas* genus than with *Schlegelella* genus as shown in Supplementary Table S1.

Based on the phylogenetic analysis a comparison was made between the characteristics of strain AK31<sup>T</sup> with the type strains of the genus *Caldimonas* (*C. manganoxidans* and *C. taiwanensis*), of the family *Comamonadaceae* (Tables 1, 2 and Supplementary Table S1) and a number of differences were observed. For instance, strain AK31<sup>T</sup> differed from both strains with respect to cell size, temperature optimum and growth range, pH growth optimum and growth range, biochemical activities, hydrolysis of complex substrates, different carbon substrate utilization, G + C content, fatty acid and polar lipid composition (Tables 1, 2 and Supplementary Table S1). Thus, the cumulative differences that strain AK31<sup>T</sup> exhibits with the above closely related type strains unambiguously supports the creation of a new species of the genus *Caldimonas* for which the name *Caldimonas meghalayensis* sp. nov. is proposed.

#### **Description of *Caldimonas meghalayensis* sp. nov.**

*Caldimonas meghalayensis* (me.gha.la.yen'sis. N.L. fem. adj. meghalayensis of or belonging to Meghalaya).

Cells are Gram-negative, non spore-forming rods (0.4-0.6 X 1.4-2.0 µm), motile with single flagellum. Colonies on nutrient agar were circular, smooth, light yellow, opaque and convex with entire margins. Grows at 25-55 °C with an optimum temperature of 45 °C and tolerates up to 2% (w/v) NaCl. Grows at pH 6-10 with an optimum pH of 7.0. Produced PHB granules. Starch and Tween 40, urea are hydrolyzed but agar, casein, cellulose, DNA, gelatin, aesculin, Tween 20, 60, 80 are not hydrolyzed. The methyl red and Voges-Proskauer's reactions are negative. Nitrate is reduced but H<sub>2</sub>S and indole are not produced. Utilises D-glucose, glycerol, lactate, citrate, tartrate, succinate, maltose, pyruvate, gluconate, malonate, 3-hydroxybutyrate but not sucrose, esculin, D-sorbitol, D-galactose, D-glucosamine, *myo*-inositol, L-arabinose, D-xylose, D-salicin, trehalose, inulin, dulcitol,

sorbose, D-mannose, D-ribose, lactose, maltose, D-mannose, L-rhamnose, erythritol, xylitol, raffinose, melibiose, propionate, 4-hydroxybutyrate, oxalate, melezitose, quinate, acetate, adonitol, D-cellobiose, D-tagatose, D-trehalose, palatinose, L-histidine, L-malate, coumarate,  $\alpha$ -methyl-D-mannoside, 5-keto-D-gluconate and glucuronate. L-pyrrolydonyl-arylamidase, L-proline arylamidase, tyrosine arylamidase and  $\alpha$ -glucosidase activities were present but Ala-Phe-Pro-arylamidase,  $\beta$ -galactosidase,  $\beta$ -N-acetylglucosaminidase,  $\gamma$ -glutamyl-transferase,  $\beta$ -xylosidase,  $\beta$ -alanine arylamidase, L-proline arylamidase, lipase, tyrosine arylamidase,  $\beta$ -N-acetylgalactosaminidase,  $\alpha$ -galactosidase, phosphatase, glycine arylamidase, ornithine decarboxylase, lysine decarboxylase,  $\beta$ -glucuronidase, glu-gly-arg-arylamidase activities were absent. Positive for succinate alkalisation. Negative for L-lactate alkalisation, ELLMAN reaction and 0/129 Resistance. Susceptible to antibiotics ( $\mu\text{g}$  per disc unless indicated) ampicillin (25), ceftazidime (30), spectinomycin (100), enoxacin (10), chloramphenicol (30), cefalexin (15), enrofloxacin (5), amoxicillin (30), ciprofloxacin (10), cefazolin (30), cefprozil (30), cephadroxil (30); resistant to vancomycin (30), tetracycline (30), bacitracin (8), novobiocin (30) and polymycin (300) and intermediate sensitivity penicillin-G (2 units), clarithromycin (15), cinoxacin (100), erythromycin (10), chlorotetracycline (30), lincomycin (2) and neomycin (30). The major cellular fatty acids are C<sub>16:0</sub>, C<sub>17:0</sub> cyclo, C<sub>16:1</sub>  $\omega$ 7c and/or iso-C<sub>15:0</sub> 2OH (summed feature 3), C<sub>10:0</sub> 3OH, C<sub>12:0</sub>, C<sub>14:0</sub> and C<sub>18:1</sub>  $\omega$ 7c. UQ-8 is the respiratory quinone and the major polar lipids consist of diphosphatidylglycerol, phosphatidylethanolamine, three unidentified phospholipids and one glycolipid. The G + C content of DNA of the strain AK31<sup>T</sup> was 66.7 mol%.

The type strain AK31<sup>T</sup> (= MTCC 11703<sup>T</sup> = JCM 18786<sup>T</sup>), was isolated from the water sample collected from the Jakrem hot spring located in Meghalaya state of North-East India. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AK31<sup>T</sup> is HF562216.

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### Figure Legends:

**Fig. 1** Negatively stained electron micrograph (bar 0.2  $\mu\text{m}$ ) of strain AK31<sup>T</sup>. The cell bears single polar flagellum and transparent intracellular granules.

**Fig. 2** The polar lipid profiles of, (a) strain AK31<sup>T</sup> and the related type strain (b) *Caldimonas manganoxidans* JCM 10698<sup>T</sup> (c) *Caldimonas taiwanensis* LMG 22827<sup>T</sup>. The polar lipids were identified as follows: diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), three unidentified phospholipids (PL) and one glycolipid (GL).

**Fig. 3** Maximum likelihood tree based on 16S rRNA gene sequences representing the phylogenetic relationships between the strain AK31<sup>T</sup> and the related type strains obtained from the database of type strains with validly published prokaryotic names at EzTaxon-e identification service. Filled circles indicate branching topologies that are common in this tree and the tree obtained using the neighbor-joining method. Numbers at the nodes are bootstrap values >50%. *Burkholderia xenovorans* LB400<sup>T</sup> (CP000270) was taken as the out group organism. The scale bar corresponds to the expected number of changes per nucleotide position.

**Supplementary Fig. S1.** Neighbor-joining tree based on 16S rRNA gene sequences representing the phylogenetic relationships between the strain AK31<sup>T</sup> and the related type strains obtained from the database of type strains with validly published prokaryotic names at EzTaxon-e identification service. *Burkholderia xenovorans* LB400<sup>T</sup> (CP000270) was taken as the out group organism. The scale bar corresponds to the expected number of changes per nucleotide position.

**Table 1** Phenotypic features that distinguish strain AK31<sup>T</sup> with the closely related type strains of *Caldimonas* species

All the strains were thermophilic, aerobic, motile, Gram-negative rods. Oxidase was positive while the methyl red and Voges-Proskauer's reactions were negative. Nitrate was reduced but H<sub>2</sub>S was not produced. Utilises D-glucose, glycerol, lactate, citrate, tartrate, succinate, pyruvate, gluconate, 3-hydroxybutyrate but not sucrose, esculin, D-sorbitol, D-galactose, D-glucosamine, *myo*-inositol, L-arabinose, D-xylose, D-salicin, trehalose, inulin, dulcitol, sorbose, D-mannose, D-ribose, lactose, D-mannose, L-rhamnose, erythritol, xylitol, raffinose, melibiose, propionate, 4-hydroxybutyrate, oxalate, melezitose, quinate, adonitol, D-cellobiose, D-tagatose, D-trehalose, palatinose, L-histidine, L-malate, coumarate,  $\alpha$ -methyl-D-mannoside, 5-keto-D-gluconate and glucuronate. Ala-Phe-Pro-arylamidase,  $\beta$ -galactosidase,  $\beta$ -N-acetylglucosaminidase,  $\gamma$ -glutamyl-transferase,  $\beta$ -xylosidase,  $\beta$ -alanine arylamidase, L-proline arylamidase, lipase, tyrosine arylamidase,  $\beta$ -N-acetylgalactosaminidase,  $\alpha$ -galactosidase, phosphatase, glycine arylamidase, ornithine decarboxylase, lysine decarboxylase,  $\beta$ -glucuronidase, glu-gly-arg-arylamidase activities were absent. Positive for succinate alkalisation. Negative for L-lactate alkalisation, 0/129 resistance and ELLMAN reaction. Data is from the present study.

Characteristic	<i>C. meghalayensis</i> AK31 <sup>T</sup>	<i>C. manganoxidans</i> JCM 10698 <sup>T</sup>	<i>C. taiwanensis</i> LMG 22827 <sup>T</sup>
Cell Size ( $\mu$ m)	0.4-0.6 x 1.4-2.0	0.5-0.7 x 2.2-3.5	0.6-0.8 x 1.2-2.2
Temperature growth range ( $^{\circ}$ C)	25-55	30-55	35-60
Temperature optimum ( $^{\circ}$ C)	45	50	55
pH growth range	6-10	6-9	6-8
pH optimum	7	8-9	7
<b>Biochemical</b>			
Oxidase	+	+	-
Catalase	+	(+)	+
Indole	-	-	+
Malonate	+	+	-
Maltose	(+)	-	-
Fructose	-	+	-
Mannitol	-	+	-
Acetate	-	-	+
Arabitol	-	+	-
$\alpha$ -glucosidase	+	+	-
$\beta$ -glucosidase	(+)	-	-
Urease	(+)	-	+
Lipase	-	-	-
L-Pyrrolydonyl-arylamidase	+	+	-
L-Proline arylamidase	+	+	-
Tyrosine arylamidase	+	+	-
Succinate Alkalinisation	+	+	-
Glutamyl arylamidase	+	-	-
L-lactate assimilation	(+)	-	-
Polar lipids	DPG, PE, GL, PL1- PL3	DPG, PE, GL, PL1-PL2	DPG, PE, GL, PL1- PL5
DNA G + C content (mol%)	66.7	66.2	65.9

+, positive; (+), weak positive; -, negative; (-), weak negative

DPG: diphosphatidylglycerol, PE: phosphatidylethanolamine, PL: phospholipids (PL), GL: glycolipid

**Table 2.** Fatty acid composition of strain AK31<sup>T</sup> with the closely related type strains of *Caldimonas* species

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. Values for fatty acid below 1.0% in the strains are not given. ND, not detected. Data is from the present study.

Fatty acid composition	<i>C. meghalayensis</i> AK31 <sup>T</sup>	<i>C. manganoxidans</i> JCM 10698 <sup>T</sup>	<i>C. taiwanensis</i> LMG 22827 <sup>T</sup>
C <sub>10:0</sub> 3OH	<b>8.0</b>	<b>7.5</b>	<b>8.0</b>
C <sub>12:0</sub>	<b>5.8</b>	<b>5.6</b>	<b>5.9</b>
C <sub>14:0</sub>	5.3	3.7	4.5
C <sub>16:0</sub>	<b>34.8</b>	<b>29.8</b>	<b>31.9</b>
C <sub>17:0</sub> cyclo	<b>27.1</b>	<b>14.3</b>	<b>16.3</b>
C <sub>17:0</sub>	1.4	3.4	1.6
C <sub>18:3</sub> ω6,9,12c	ND	ND	1.4
C <sub>18:1</sub> ω7c	<b>5.3</b>	<b>17.6</b>	<b>11.5</b>
C <sub>20:2</sub> ω6,9c	ND	ND	1.4
Summed Feature 3 <sup>a</sup>	<b>9.6</b>	<b>18.2</b>	<b>13.1</b>

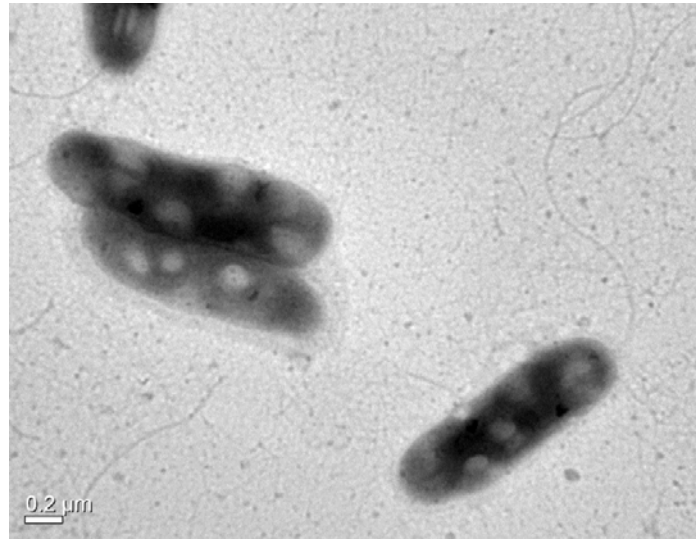
Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system.

<sup>a</sup>Summed feature 3 comprises C<sub>16:1</sub> ω7c and/or iso-C<sub>15:0</sub> 2OH.

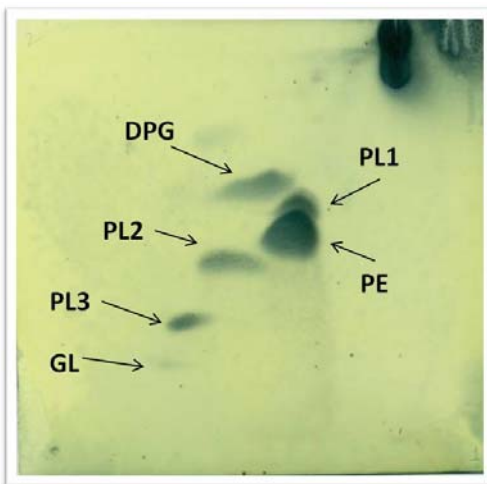
**Supplementary Table S1** Distinguishing characteristics of strain AK31<sup>T</sup> with genus level characteristics of *Caldimonas* and *Schlegelella*

Characteristic	<i>C. meghalayensis</i> AK31 <sup>T</sup>	<i>Caldimonas</i>	<i>Schlegelella</i>
Cell Size (μm)	0.4-0.6 x 1.4-2.0	0.5-0.7 x 2.2-3.5	1.0-2.8X 0.5-0.6
Major Fatty Acid	C <sub>16:0</sub> , C <sub>17:0</sub> cyclo and Summed Feature 3 <sup>a</sup>	C <sub>16:0</sub> , C <sub>17:0</sub> cyclo and Summed Feature 3 <sup>a</sup>	C <sub>16:0</sub> and C <sub>17:0</sub> cyclo
DNA G + C content (mol%)	66.7	65-66	69-70

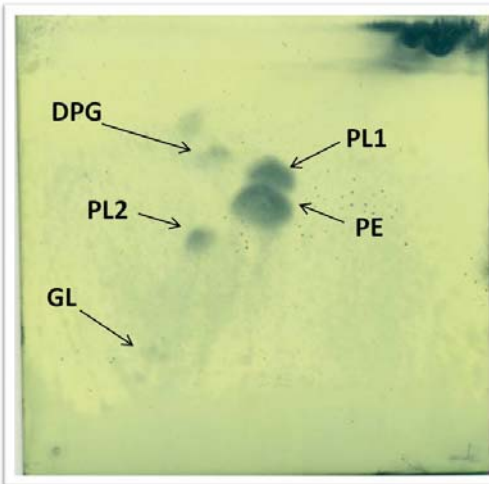
Data for genus level description of *Caldimonas* is taken from Takeda et al. 2002 and genus level description of *Schlegelella* is taken from Elbanna et al. 2003.



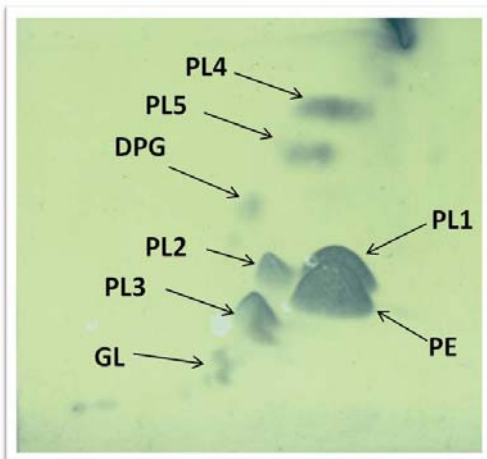
**Fig. 1**



(a)



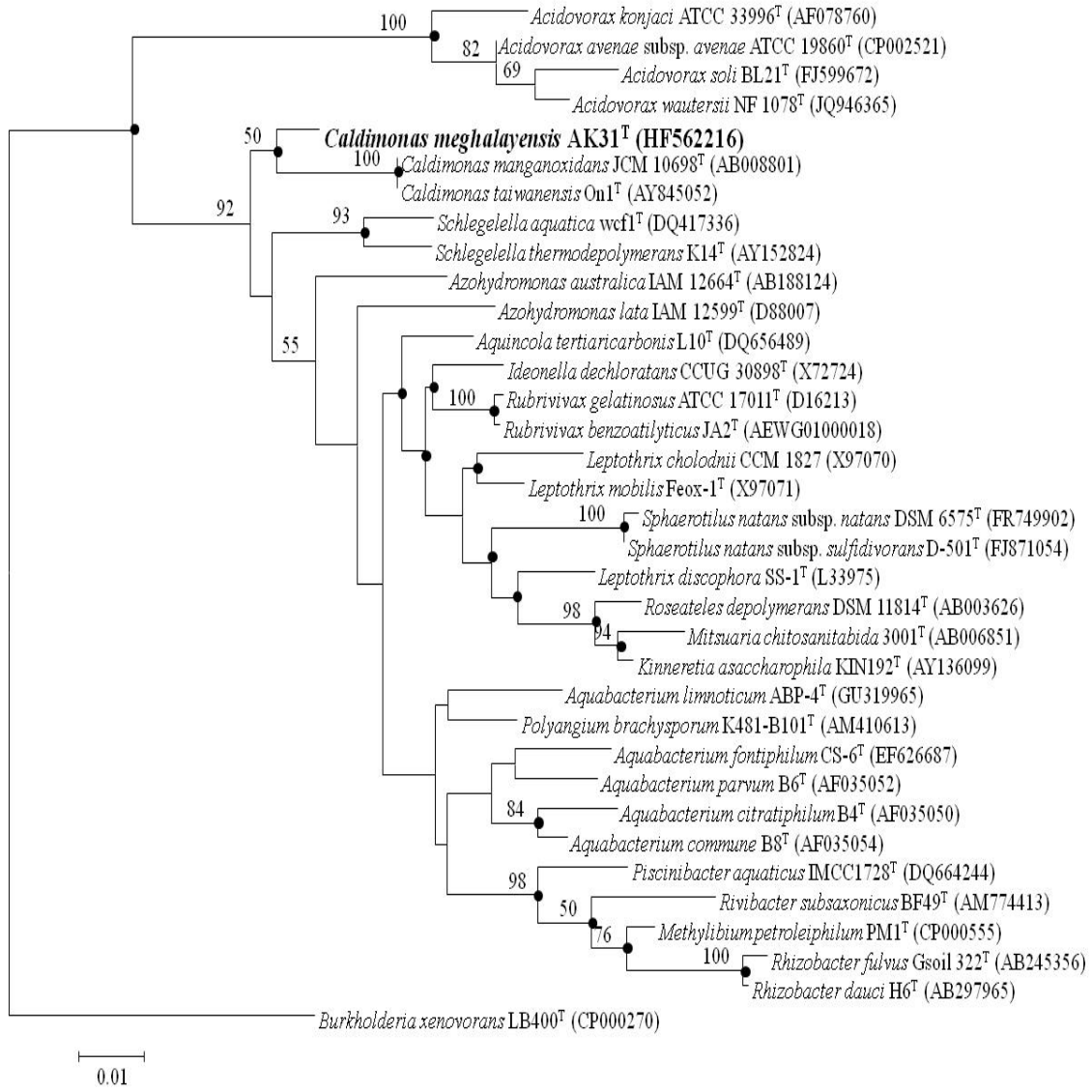
(b)



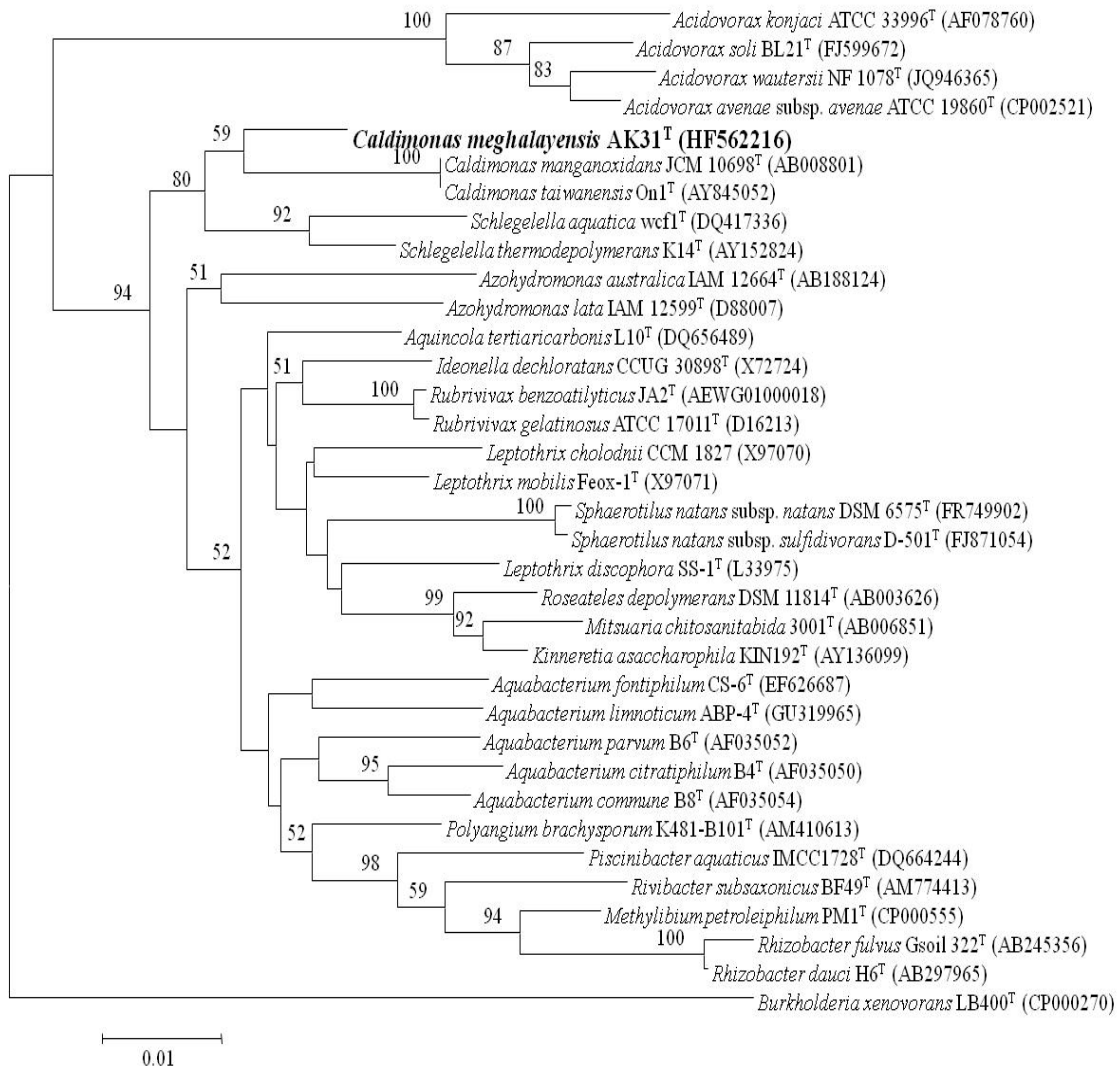
(c)

**Fig. 2**





**Fig. 3**



**Supplementary Fig. S1**