

## *Melaminivora alkalimesophila* gen. nov., sp. nov., a melamine-degrading betaproteobacterium isolated from a melamine-producing factory

Han Wang,<sup>1,2†</sup> Jiangwei Li,<sup>1†</sup> Anyi Hu,<sup>1</sup> Dan Qin,<sup>1</sup> Heli Xu<sup>1</sup> and Chang-Ping Yu<sup>1</sup>

Correspondence  
Chang-Ping Yu  
cpyu@iue.ac.cn

<sup>1</sup>Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, PR China

<sup>2</sup>College of Ecology and Resources Engineering, Wuyi University, Wuyishan City 354300, PR China

A taxonomic study was carried out on strain CY1<sup>T</sup>, which is a novel bacterium isolated from wastewater sludge of a melamine-producing factory in Sanming city, Fujian, China. Strain CY1<sup>T</sup> was shown to rapidly and completely degrade melamine to NH<sub>3</sub> and CO<sub>2</sub> under aerobic conditions. The isolate was Gram-stain-negative, short-rod-shaped and motile by one unipolar flagellum. Growth was observed at salinities from 0 to 7% NaCl (optimum, 0.1%), at temperatures from 15 to 50 °C (optimum, 40–45 °C) and at pH 7–9.5 (optimum pH 9.5). Quinone-8 was detected as the major respiratory quinone. 16S rRNA gene sequence comparisons showed that strain CY1<sup>T</sup> was affiliated to the family *Comamonadaceae* in the class *Betaproteobacteria*. It was most closely related to members of the genera *Alicyclophilus* (95.5%), *Diaphorobacter* (94.6–95.1%), *Acidovorax* (92.9–95.4%), *Delftia* (93.0–93.6%) and *Comamonas* (92.6–93.9%). The average nucleotide identity (ANI) values between strain CY1<sup>T</sup> and those representing related genera ranged from 84.0 to 86.1% using Mummer, and from 74.9 to 81.1% using BLAST. The dominant fatty acids were C<sub>16:1ω7c</sub> and/or C<sub>16:1ω6c</sub>, C<sub>16:0</sub>, C<sub>10:0</sub> 3-OH and C<sub>18:1ω7c</sub> and/or C<sub>18:1ω6c</sub>, and the major polar lipids consisted of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, one unidentified phospholipid and one unidentified aminophospholipid. The G + C content of the chromosomal DNA was 69.5 mol%. On the basis of the phenotypic and phylogenetic data, strain CY1<sup>T</sup> represents a novel species of a new genus, for which the name *Melaminivora alkalimesophila* gen. nov., sp. nov. is proposed. The type strain of *Melaminivora alkalimesophila* is CY1<sup>T</sup> (=CCTCC AB 2012024<sup>T</sup>=DSM 26006<sup>T</sup>).

Melamine (2,4,6-triamino-1,3,5-triazine) is a member of the *s*-triazine family. It was developed in the 1830s and is widely used in the industrial production of many laminates, plastics, adhesives, countertops, kitchenware and whiteboards (Wittcoff *et al.*, 2004). Although melamine has been previously considered to have low toxicity to mammals, recent studies suggest that melamine may be an emerging contaminant due to a number of widely publicized food-safety incidents throughout the world

(Ingelfinger, 2008). A few bacteria, such as *Rhodococcus corallinus* NRRL B-15444R (Jutzi *et al.*, 1982), *Klebsiella terrigena* DRS-1 (Shelton *et al.*, 1997), *Acidovorax citrulli* NRRL B-12227 (Karns, 1999), *Nocardioides* sp. ATD6 (Takagi *et al.*, 2012) and *Microbacterium esteraromaticum* MEL1 (Shiomi & Ako, 2012), have been reported to degrade melamine. Here, we described the taxonomic characterization of a novel melamine-degrading strain, CY1<sup>T</sup>, which was isolated from wastewater sludge of a melamine-producing factory in Sanming city, Fujian, China.

†Han Wang and Jiangwei Li contributed equally to this work.

Abbreviation: ANI, average nucleotide identity.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Melaminivora alkalimesophila* CY1<sup>T</sup> is JQ676982. The draft genome sequence of strain CY1<sup>T</sup> has been deposited under accession number ALEE00000000.

Two supplementary figures and one supplementary table are available with the online version of this paper.

The wastewater sludge was sampled at site no. 6 (117° 16.097' E 26° 37.193' N) in Sanming city, Fujian province, PR China, in May 2010. The sample was enriched in nitrate-free NMS medium (Yu *et al.*, 2007), at pH 7.3–7.5, with melamine (500 mg l<sup>-1</sup>) as the carbon and nitrogen source. Enrichment of melamine-degrading consortia was conducted immediately after sampling, at 30 °C

and 150 r.p.m. Sequential transfers were performed five times at intervals of 1 week. Melamine-degrading bacteria were isolated according to their melamine degrading ability. For morphological and biochemical characterization, strain CY1<sup>T</sup> was subcultured on R<sub>2</sub>A medium (Hope Bio-Technology). The medium was sterilized at 121 °C for 20 min. Characterization and classification of strain CY1<sup>T</sup> were done with polyphasic methods.

Genomic DNA was prepared using a TIANamp Bacteria DNA kit (Tiangen Biotech), and the 16S rRNA gene was amplified by PCR using universal primers 16SF and 16SR (Liu & Shao, 2005). Sequences of related taxa were obtained from the GenBank database. Phylogenetic analysis was performed using MEGA version 4 (Tamura *et al.*, 2007) after multiple alignment of data by DNAMAN (version 5.1; Lynnon Biosoft). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method (Saitou & Nei, 1987) was determined by using bootstrap values based on 1000 replications.

A nearly full-length 16S rRNA gene sequence (1436 bp) of strain CY1<sup>T</sup> was determined, and the sequence information was also confirmed by the whole genome sequencing result described below. Phylogenetic analysis indicated that strain CY1<sup>T</sup> formed a distinct evolutionary lineage within the family *Comamonadaceae* in the class *Betaproteobacteria*. (Fig. 1). Closely related genera were *Alicyclophilus* (95.5% 16S rRNA gene sequence similarity), followed by *Diaphorobacter* (94.6–95.1%), *Acidovorax* (92.9–95.4%), *Delftia* (93.0–93.6%) and *Comamonas* (92.6–93.9%). 16S rRNA gene sequence divergence between strain CY1<sup>T</sup> and all recognized species was greater than 4.5% and the distinct phylogenetic relationships revealed that strain CY1<sup>T</sup> could not be assigned to any of the recognized genera. Consequently, strain CY1<sup>T</sup> should be considered to represent a novel species of a new genus in the family *Comamonadaceae*.

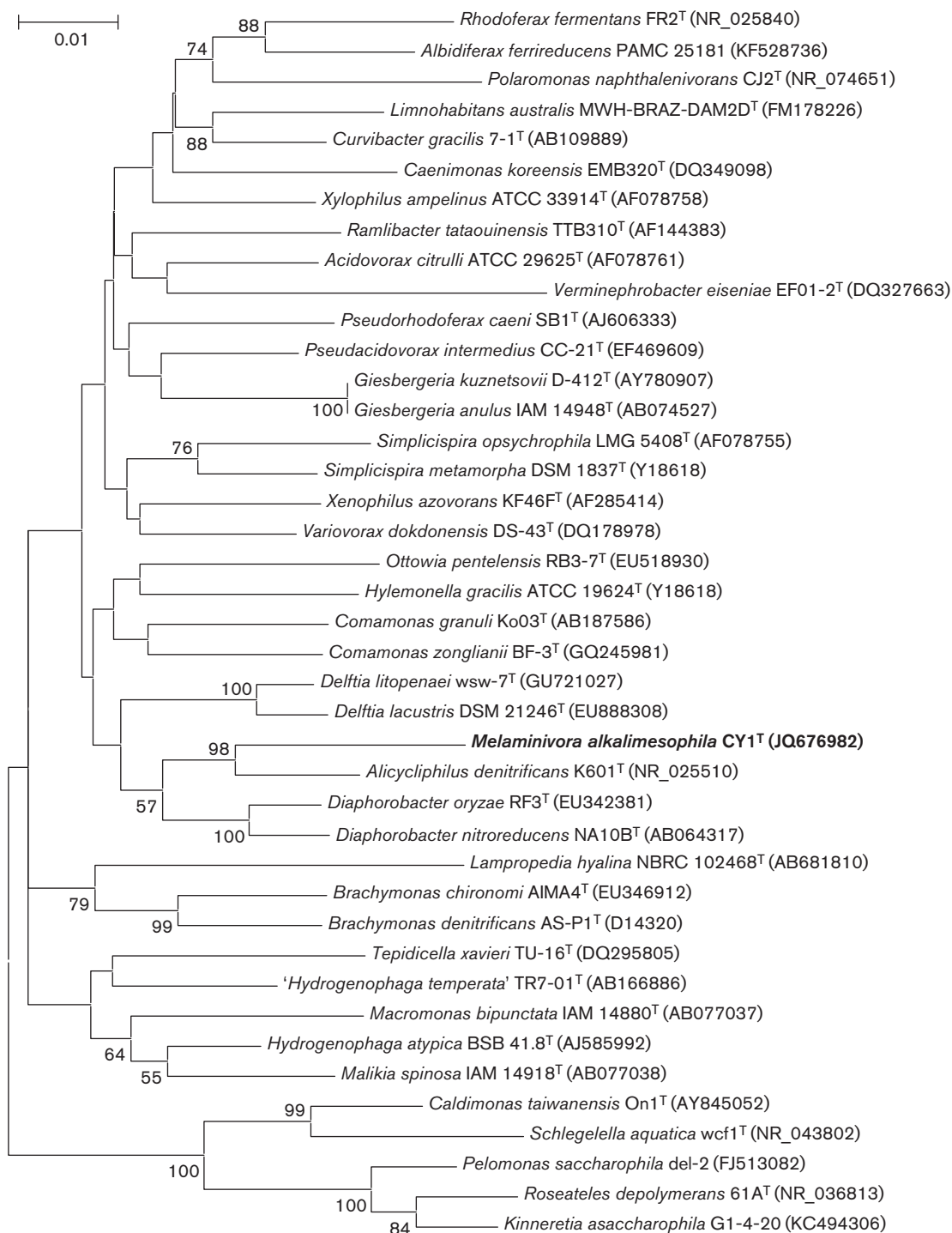
General cell morphology was studied under an Olympus inverted microscope using a 1-day-old culture of strain CY1<sup>T</sup> grown on R<sub>2</sub>A agar. The motility of the strain was tested using the semi-solid agar method (Tittsler & Sandholzer, 1936). For electron microscopy, exponential-phase cells were harvested, subsequently suspended and adsorbed on a Formvar-carbon-coated grid, then stained with phosphotungstic acid (Fig. S1, available in the online Supplementary Material). Gram stain, catalase and oxidase activities were carried out according to Dong & Cai (2001). The optimal growth temperature was determined over the temperature range 4–60 °C using R<sub>2</sub>A medium. The pH range for growth was examined at 30 °C in the same medium over the range pH 3.0–12.0 (pH adjusted with HCl and NaOH). Tolerance of NaCl was tested by using R<sub>2</sub>A medium supplemented with NaCl concentrations of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.5, 2, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0% (w/v). Antibiotic susceptibility tests were performed by the disc diffusion method as described by Shieh *et al.* (2003). Oxoid discs

were used in this study, and a zone  $\geq 8$  mm indicated resistance. Melamine (initial concentration 500 p.p.m.) degradation was detected at 30 °C in 120 h using Dionex UltiMate 3000 HPLC. Other biochemical tests were carried out using API 20NE, API ZYM (bioMérieux) and Biolog GN2 kits according to the manufacturers' instructions, with the adjustment of all NaCl concentrations to be 0.1%. *Alicyclophilus denitrificans* K601<sup>T</sup> was tested at the same time for comparison. These results are given in the species description and Table 1.

The G+C content of the chromosomal DNA was determined according to methods described by Mesbah & Whitman (1989) using HPLC. The DNA G+C content of the new isolate, CY1<sup>T</sup>, was 69.5 mol% and was close to that previously reported for *Alicyclophilus denitrificans* K601<sup>T</sup> (66 mol%) and other members of the family *Comamonadaceae*.

Average nucleotide identity (ANI) was calculated as an alternative to DNA–DNA hybridization as described by Richter & Rosselló-Móra (2009). Whole genome DNA from strain CY1<sup>T</sup> was extracted using a DNeasy Blood & Tissue kit (Qiagen). Sequencing was performed using an Illumina Solexa GAI instrument with a paired-end library (500 bp). A total of 556.8 Mbp of sequence was produced, providing approximately 185-fold coverage. Genome sequences were assembled *in silico* using Velvet 1.2.06 (Zerbino & Birney, 2008). Whole genome sequences from 13 strains representing the most closely related genera, *Acidovorax*, *Alicyclophilus*, *Delftia*, *Comamonas* and *Pseudacidovorax*, including *Acidovorax* sp. KKS102 (NC\_018708), *Acidovorax avenae* subsp. *avenae* ATCC 19860<sup>T</sup> (NC\_015138), '*Acidovorax ebreus*' TPSY (NC\_011992), *Acidovorax citrulli* AAC00-1 (NC\_008752), *Acidovorax* sp. JS42 (NC\_008782), *Alicyclophilus denitrificans* K601<sup>T</sup> (NC\_015422), *Alicyclophilus denitrificans* BC (NC\_014910), *Delftia acidovorans* CCUG 15835 (NZ\_AGGY01000000), *Delftia* sp. Cs1-4 (NC\_015563), *Comamonas testosteroni* ATCC 11996<sup>T</sup> (NZ\_AHIL01000000), *Comamonas composti* DSM 21721<sup>T</sup> (AUCQ00000000) and *Comamonas badia* DSM 17552<sup>T</sup> (AXVM01000000), as well as *Pseudacidovorax intermedius* NH-1 (NZ\_ANOY00000000) were retrieved from the GenBank database. The ANI analysis was carried out using the software JSpecies v1.2.1 (Richter & Rosselló-Móra, 2009). As can be seen in Table 2, ANI values of the 13 strains ranged from 84.0 to 86.1% using Mummer, and from 74.9 to 81.1% using BLAST. In all cases, the ANI results were far below the threshold of 94–96% that would correspond to the species borderline (Richter & Rosselló-Móra, 2009), indicating a very low taxonomic relatedness between strain CY1<sup>T</sup> and the reference genera. All these results suggested the new strain represents a new genus within the family *Comamonadaceae*.

Fatty acids in whole cells grown on R<sub>2</sub>A medium at 28 °C for 48 h (exponential phase) were extracted, saponified and esterified; this was followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Sasser, 1990). The fatty acid profile of its closest



**Fig. 1.** Neighbour-joining tree showing the phylogenetic positions of strain CY1<sup>T</sup> and representatives of other members of the family Comamonadaceae, class Betaproteobacteria, based on 16S rRNA gene sequences. Bootstrap values (>50%, expressed as percentages of 1000 replications) are shown at branch points. Bar, 0.01 nucleotide substitution rate ( $K_{nuc}$ ) units.

phylogenetic relative, *Alicycliphilus denitrificans* K601<sup>T</sup>, was determined in parallel with that of strain CY1<sup>T</sup> in this study. As shown in Table S1, the presence of  $C_{16:0}$ , summed feature 3 ( $C_{16:1\omega7c}$  and/or  $C_{16:1\omega6c}$ ) and

summed feature 8 ( $C_{18:1\omega7c}$  and/or  $C_{18:1\omega6c}$ ) as the major fatty acids of strain CY1<sup>T</sup> was similar to related genera. However, strain CY1<sup>T</sup> showed a relatively higher content (>10%) of  $C_{10:0}$  3-OH, which is lower ( $\leq 6\%$ ) in

**Table 1.** Characteristics that differentiate CY1<sup>T</sup> from related genera

Taxa: 1, strain CY1<sup>T</sup> (this study); 2, *Alicyclophilus* (data from Mechichi *et al.*, 2003); 3, *Diaphorobacter* (Pham *et al.*, 2009; Khan & Hiraishi, 2002); 4, *Acidovorax* (Willems *et al.*, 1990; Li *et al.*, 2011); 5, *Pseudacidovorax* (Kämpfer *et al.*, 2008); 6, *Delftia* (Wen *et al.*, 1999); 7, *Comamonas* (Tamaoka *et al.*, 1987). All taxa were positive for oxidase. Characteristics are scored as: w, weakly positive; +, positive; -, negative. NA, No data available; d, variable.

Characteristic	1	2	3	4	5	6	7
Cell morphology	Rods	Rods	Rods	Rods	Rods	Rods	Rods /spirilla
Flagellation	Unipolar	Polar	Unipolar	Unipolar/absent	Unipolar	Polar/bipolar tufts	Polar/bipolar tuft
Denitrification	-	+*	+	d	-	-	-
Nitrate reduction	+	+*	+	+	-	+	NA
Catalase	+	+*	+	d	+	+	+
Urease	+	+*	+	d	NA	NA	d
Degradation of melamine	+	-*	-*	-/+†	NA	NA	NA
Optimum temperature for growth (°C)	40-45	28-30	28-35	25-28	30	20-35‡	NA
Optimum pH for growth	9.5	7.2-7.4	7-8	7.0-7.5	7.1-7.5	6.0-7.0‡	NA
DNA G+C content (mol%)	69.5	66	64-65	64-65	70.1	67-69	61-67.1
Major fatty acids (>8%)	C <sub>16:1</sub> ω7c and/or C <sub>16:1</sub> ω6c, C <sub>16:0</sub> , C <sub>18:1</sub> ω7c and/or C <sub>18:1</sub> ω6c, C <sub>10:0</sub>	C <sub>16:1</sub> ω7c and/or C <sub>16:1</sub> ω6c, C <sub>16:0</sub> , C <sub>17:0</sub> cyclo, C <sub>18:1</sub> ω7c and/or C <sub>18:1</sub> ω6c*	C <sub>16:1</sub> ω7c and/or C <sub>16:1</sub> ω6c, C <sub>16:0</sub> , C <sub>18:1</sub> ω7c and/or C <sub>18:1</sub> ω6c	C <sub>16:1</sub> ω7c and/or C <sub>16:1</sub> ω6c, C <sub>16:0</sub> , C <sub>18:1</sub> ω7c and/or C <sub>18:1</sub> ω6c	C <sub>16:1</sub> ω7c and/or C <sub>16:1</sub> ω6c, C <sub>16:0</sub> , C <sub>17:0</sub> cyclo, C <sub>18:1</sub> ω7c and/or C <sub>18:1</sub> ω6c	C <sub>16:0</sub> , C <sub>16:1</sub> ω7c and/or C <sub>16:1</sub> ω6c, C <sub>18:1</sub> ω7c and/or C <sub>18:1</sub> ω6c	C <sub>16:0</sub> , C <sub>16:1</sub> ω7c and/or C <sub>16:1</sub> ω6c, C <sub>18:1</sub> ω7c and/or C <sub>18:1</sub> ω6c
Major quinone(s)	3-OH Q-8	ω6c*	Q-8	Q-8	ω6c	Q-8	Q-8
Carbon source							
Citric acid	-	-*	-	-	w	+	d
D-Fructose	-	-*	w	d	w	+	-
D-Glucose	-	-*	-/+§	d	w	-	-
D-Mannitol	-	-*	-	d	-	+	-
Melibiose	-	-*	-§	-	-	-	NA
D-Sorbitol	-	-*	-	d	-	-	NA
Itaconic acid	-	w*	+/-§	-	-	+	d
L-Arabinose	-	-*	-§	d	+	-	-
L-Leucine	w	w*	-	d	-	+	NA
L-Rhamnose	-	-*	-§	-	-	-	NA
L-Serine	-	+*	-/+§	d	-	-	-
Maltose	-	-*	-§	-	-	-	-
Tween 40	-	+*	NA	+	NA	NA	NA
Tween 80	-	+*	-	+	NA	+	NA
γ-Aminobutyric acid	-	+*	NA	d	w	NA	NA

\*Data from this study.

†Only positive for *Acidovorax citrulli* NRRL B-12227<sup>T</sup> (Karns, 1999).

‡Data were adapted from Chen *et al.* (2012).

§Data were adapted from Pham *et al.* (2009).

**Table 2.** ANI analysis between strain CY1<sup>T</sup> (sequenced in this study) and 13 reference strains of the most closely related genera (obtained from public repositories)

ANiB, analysis using BLAST; ANIm, analysis using Mummer.

Genus	Species	CY1 <sup>T</sup>	
		ANiB (%)	ANIm (%)
<i>Acidovorax</i>	' <i>Acidovorax ebreus</i> ' TPSY	80.21	85.67
	<i>Acidovorax citrulli</i> AAC00-1	78.09	84.81
	<i>Acidovorax</i> sp. KKS102	76.76	84.04
	<i>Acidovorax</i> sp. JS42	80.27	85.83
	<i>Acidovorax avenae</i> subsp. <i>avenae</i> ATCC 19860 <sup>T</sup>	78.09	84.70
<i>Alicyclophilus</i>	<i>Alicyclophilus denitrificans</i> K601 <sup>T</sup>	81.07	86.06
	<i>Alicyclophilus denitrificans</i> BC	81.06	86.03
<i>Delftia</i>	<i>Delftia acidovorans</i> CCUG 15835	77.64	84.76
	<i>Delftia</i> sp. Cs1-4	77.36	84.44
<i>Comamonas</i>	<i>Comamonas testosteroni</i> ATCC 11996 <sup>T</sup>	75.32	83.62
	<i>Comamonas composti</i> DSM 21721 <sup>T</sup>	76.24	84.04
	<i>Comamonas badia</i> DSM 17552 <sup>T</sup>	76.83	84.42
<i>Pseudacidovorax</i>	<i>Pseudacidovorax intermedius</i> NH-1	74.91	84.23

*Alicyclophilus denitrificans* K601<sup>T</sup> (this study) and members of the genera *Diaphorobacter* (Khan & Hiraishi, 2002), *Acidovorax* (Willems *et al.*, 1990), *Delftia* (Chen *et al.*, 2012), *Comamonas* (Tamaoka *et al.*, 1987) and *Pseudacidovorax* (Kämpfer *et al.*, 2008). The variation in the proportion of C<sub>17:0</sub> cyclo and summed feature 3 (C<sub>16:1</sub>ω6c and/or C<sub>16:1</sub>ω7c), as well as the absence of C<sub>20:0</sub> and summed feature 7 (unknown equivalent chain length 18.846 and/or C<sub>19:1</sub>ω6c), indicated significant differences of strain CY1<sup>T</sup> from the genus *Alicyclophilus*. In addition, we also observed large differences in the proportion of summed feature 8 (C<sub>18:1</sub>ω7c and/or C<sub>18:1</sub>ω6c) between strain CY1<sup>T</sup> and the members of the genera *Diaphorobacter*, *Acidovorax*, *Delftia*, *Comamonas* and *Pseudacidovorax*. Strain CY1<sup>T</sup> showed higher amounts of C<sub>17:0</sub> cyclo compared with members of the genera *Diaphorobacter* and *Acidovorax*. The presence of C<sub>12:0</sub> and the absence of C<sub>18:1</sub> 2-OH and C<sub>16:1</sub> 2-OH distinguished strain CY1<sup>T</sup> from the genus *Pseudacidovorax*.

Quinones were extracted, fractionated, and analysed by using reversed-phase HPLC as described by Komagata & Suzuki (1987), and the HPLC results showed that quinone-8 (Q-8) accounted for nearly 100% of the total quinone content of this new isolate.

Polar lipids were extracted and fractionated using the method described by Klein *et al.* (2009), and the analysis of polar lipids was carried out by the Identification Service of the DSMZ, Braunschweig, Germany, using a standard procedure (Tindall, 1990a, b). The major polar lipids consisted of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, one unidentified phospholipid and one unidentified aminophospholipid (Fig. S2).

Based on 16S rRNA gene sequence analysis, strain CY1<sup>T</sup> was classified in the family *Comamonadaceae* of the class

*Betaproteobacteria* (Willems *et al.*, 1991). The presence of C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c and C<sub>16:0</sub> as the major components of whole-cell fatty acids and Q-8 as the major respiratory quinone justify its placement in this family. In addition, this classification was also confirmed by the DNA G+C value (69.5 mol%), which is within the range of those for this family (57–70 mol%). Phenotypically, this new isolate was similar to some genera of the family *Comamonadaceae* (*Alicyclophilus*, *Diaphorobacter*, *Acidovorax*, *Delftia*, *Comamonas* and *Pseudacidovorax*), but the 16S rRNA gene sequence divergences between strain CY1<sup>T</sup> and the genera within the family *Comamonadaceae* were all greater than 4.5% and the distinct phylogenetic relationships revealed that strain CY1<sup>T</sup> could not be assigned to any of the existing genera. Furthermore, the divergences were confirmed by the results of ANI analysis, and the ANI values (ranging from 84.0 to 86.1% using Mummer, and from 74.9 to 81.1% using BLAST) between strain CY1<sup>T</sup> and related genera were far below the threshold of 94–96% that would correspond to the species borderline. Besides, from the results of the physiological characterization (Table 1), we found the optimum temperature and pH for growth of strain CY1<sup>T</sup>, the capacity for melamine degradation, the composition of major fatty acids (in particular the C<sub>10:0</sub> 3-OH), and the capability to reduce nitrate to nitrite, but not to reduce nitrate to nitrogen, as useful key characteristics for identification, which differentiated the genus *Melaminivora* gen. nov. from the genera *Alicyclophilus*, *Diaphorobacter*, *Acidovorax*, *Comamonas*, *Delftia* and *Pseudacidovorax*. Different phenotypic characteristics and substrates assimilated or utilized as sole carbon and energy sources are indicated in Table 1, which also supports the above inference. On the basis of morphological, physiological and chemotaxonomic characteristics, together with data from 16S rRNA gene sequence and whole genome sequence

comparison described above, strain CY1<sup>T</sup> represents a novel genus and species within the family Comamonadaceae, for which a name *Melaminivora alkalimesophila* gen. nov., sp. nov. is proposed.

### Description of *Melaminivora* gen. nov.

*Melaminivora* (Me.la.mi.ni.vo'ra. N.L. neut. n. *melaminum* melamine; L. v. *voro* to eat, to devour; N.L. fem. n. *Melaminivora* melamine eating).

Members are Gram-negative, oxidase-positive, catalase-positive, short rods, motile by one unipolar flagellum. Capable of reducing nitrate to nitrite, but incapable of reducing nitrate to nitrogen. The predominant fatty acids are C<sub>16:1</sub>ω7c and/or C<sub>16:1</sub>ω6c, C<sub>16:0</sub>, C<sub>10:0</sub> 3-OH and C<sub>18:1</sub>ω7c and/or C<sub>18:1</sub>ω6c, and the major polar lipids consist of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, one unidentified phospholipid and one unidentified aminophospholipid. Quinone-8 is the predominant respiratory quinone. The genus is affiliated to the family Comamonadaceae in the class Betaproteobacteria and currently contains only the type species, *Melaminivora alkalimesophila*.

### Description of *Melaminivora alkalimesophila* sp. nov.

*Melaminivora alkalimesophila* [al.ka.li.me.so'phi.la. N.L. n. *alkali* (from Arabic *al-qalyi* the ashes of saltwort) soda ash; Gr. adj. *mesos* middle; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *alkalimesophila* loving alkaline and mesophilic conditions].

Displays the following properties in addition to those given in the genus description. Cells are 2.0–3.0 μm long and 0.7–0.9 μm wide. Positive for urease, arginine dihydrolase, indole production and melamine degradation, but negative for β-galactosidase, β-glucosidase, D-glucose fermentation, gelatin hydrolysis, and use of adipic acid, capric acid, D-glucose, maltose, D-mannitol, D-mannose, L-arabinose, malic acid, N-acetylglucosamine, phenylacetic acid, potassium gluconate and trisodium citrate as a carbon source, determined by API 20NE. On R<sub>2</sub>A agar, produces smooth translucent colonies with regular edges that are 0.1–0.2 mm in diameter after 3 days of incubation at 30 °C, and slightly raised in the centre. Able to grow between pH 7 and 9.5 (optimum pH 9.5), and with 0 to 7% NaCl (optimum 0.1%) at 15–50 °C (optimum 40–45 °C), but not at 15 °C or 50 °C within 96 h. Sensitive to ceftriaxone (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), doxycycline (30 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), minocycline (30 μg), ofloxacin (5 μg), piperacillin (100 μg), polymyxin (300 μg), streptomycin (10 μg), tetracycline (30 μg) and trimethoprim (25 μg). Resistant to ampicillin (10 μg), carbenicillin (100 μg), cefalexin (30 μg), cefazolin (30 μg), cefradine (30 μg), clindamycin (2 μg), lincomycin (2 μg), metronidazole (5 μg), ofloxacin (10 μg), oxacillin (1 μg), vancomycin (30 μg), cefoperazone (75 μg), norfloxacin (10 μg) and

rifampicin (5 μg). In API ZYM tests, positive for esterase (C4), leucine aminopeptidase, naphthol-AS-BI-phosphatase and valine aminopeptidase; weakly positive for acid phosphatase, alkaline phosphatase, cystine aminopeptidase, esterase lipase (C8) and lipase (C14); negative for N-acetyl-β-glucosaminidase, trypsin, α-chymotrypsin, α-fucosidase, α-galactosidase, α-glucosidase, α-mannosidase, β-galactosidase, β-glucosidase and β-glucuronidase. Among the 95 carbon sources in the Biolog system, positive for DL-lactic acid, L-alanine, L-glutamic acid and L-proline; weakly positive for L-alanyl glycine, L-asparagine, L-leucine, L-phenylalanine, L-pyroglutamic acid, methyl pyruvate, monomethyl succinate, propionic acid, succinic acid and β-hydroxybutyric acid; negative for α-cyclodextrin, 2,3-butanediol, 2-aminoethanol, acetic acid, adonitol, bromosuccinic acid, *cis*-aconitic acid, citric acid, DL-carnitine, D-alanine, D-arabitol, cellobiose, dextrin, D-fructose, D-galactonic acid lactone, D-galactose, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, DL-α-glycerol phosphate, D-mannitol, D-mannose, melibiose, D-psicose, raffinose, D-saccharic acid, D-serine, D-sorbitol, trehalose, formic acid, gentiobiose, glucose-1 phosphate, glucose-6 phosphate, glucuronamide, glycerol, glycogen, glycyl-L-aspartic acid, glycyl-L-glutamic acid, hydroxy-L-proline, i-erythritol, inosine, itaconic acid, lactulose, L-alaninamide, L-arabinose, L-aspartic acid, L-fucose, L-histidine, L-ornithine, L-rhamnose, L-serine, L-threonine, malonic acid, maltose, *myo*-inositol, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, phenylethylamine, *p*-hydroxyphenylacetic acid, putrescine, quinic acid, sebacic acid, succinic acid, sucrose, thymidine, turanose, Tween 40, Tween 80, uridine, urocanic acid, xylitol, α-D-glucose, α-lactose, α-hydroxybutyric acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, methyl β-D-glucoside, γ-amino butyric acid and γ-hydroxybutyric acid, using Biolog GN2.

The type strain, CY1<sup>T</sup> (=CCTCC AB 2012024<sup>T</sup>=DSM 26006<sup>T</sup>) was isolated from wastewater sludge of a melamine-producing factory in Sanming city, Fujian, China. The G + C content of the DNA of the type strain is 69.5 mol%.

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