Melaminivora

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KEYWORDS: *Melaminivora*; aerobe; chemo-organotroph; melamine degradation;

2. ABSTRACT:

Rods 2.0 – 3.5 µm long and 0.5 – 0.9 µm wide. **Motile** by a single unipolar flagellum. **Gram-negative**. Nonsporulating. **Aerobe**. Nitrate is reduced to nitrite. Mesophilic, with the ability to grow between 15 and 50 ºC, pH 6-9.5 and at NaCl concentrations up to 7%. **Chemo-organotroph**. Catalase- and cytochrome c oxidase positive. The respiratory quinone is **ubiquinone 8.** Major fatty acids are summed feature 3 ($C_{16:1}$ $\omega 7c$ / iso-$C_{15:0}$ 2-OH), $C_{16:0,}$ $C_{18:1}$ $\omega 7c$. $C_{10:0}$ 3-OH is also present. Predominant polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol.

3. DEFINING PUBLICATION:

Melaminivora, Wang, Li, Hu, Qin, Xu and Yu, 2014, 1943*VP*

4. ETYMOLOGY:

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].

5. GENERIC DEFINITION:

Rods 2.0 – 3.5 µm long and 0.5 – 0.9 µm wide. **Motile** by a single unipolar flagellum. **Gram-negative**. Nonsporulating. **Aerobe**. Nitrate is reduced to nitrite. Mesophilic, with the ability to grow between 15 and 50 ºC, pH 6-9.5 and at NaCl concentrations up to 7%. **Chemo-organotroph**. Catalase- and cytochrome c oxidase positive. The respiratory quinone is **ubiquinone 8.** Major fatty acids are summed feature 3 ($C_{16:1}$ $\omega 7c$ / iso-$C_{15:0}$ 2-OH), $C_{16:0,}$ $C_{18:1}$ $\omega 7c$. $C_{10:0}$ 3-OH is also present. Predominant polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol.

The DNA G+C content (mol %) is 69.5-69.6 (HPLC).

Type species: *Melaminivora alkalimesophila*, Wang, Li, Hu, Qin, Xu and Yu, 2014, 1943^{VP}

Number of species with validated names: 2.

6. FAMILY CLASSIFICATION:

Comamonadaceae (fbm00182)

7. FURTHER DESCRIPTIVE INFORMATION:

7.1. Cell morphology:

Two species are validly named within the genus *Melaminivora*: *Melaminivora alkalimesophila* and *Melaminivora jejuensis* (Wang et al., 2014b, Kim et al., 2018), both described based on a single strain. Cells are non-spore forming Gram-negative rods, motile by a single polar flagellum. The cells of the type strains of *Melaminivora alkalimesophila* and *Melaminivora jejuensis* are 2.0–3.0 / 2.0–3.5 µm long and 0.7–0.9 / 0.5–0.7 µm wide, respectively.

7.2. Colonial and cultural characteristics:

After 3 days of incubation at 30 ºC on R2A agar, the type strain of *Melaminivora alkalimesophila* forms translucent, smooth colonies with regular edges and slightly raised in the center (0.1–0.2 mm diameter). On tryptic soy agar (TSA), the type strain of *Melaminivora jejuensis* produces pale yellow, circular, rough colonies (up to 1.5 mm diameter) after 3 days incubation at 30 ºC.

7.3. Nutrition and growth conditions:

The type strains of *Melaminivora* grow in complex media such as R2A or TSA. *Melaminivora alkalimesophila* strain CY1^T grows in a temperature range between 15 and 50 ºC, at pH 7-9.5 and at NaCl concentrations up to 7%. Optimal growth occurs between 40 and 45 ºC, pH 9.5 and 0.1% (w/v) NaCl. *Melaminivora jejuensis* strain KBB12^T grows in a temperature range of 15-45 ºC, pH 6-9 and at NaCl concentrations up to 1%, with optima at 30-37 ºC, and pH 7-8.

7.4. Metabolism:

The type strains of *Melaminivora* species are aerobic. Nitrate is reduced to nitrite, which is not further reduced to nitrogen. Both are chemo-organotrophs able to assimilate a set of single carbon sources, although a distinct nutritional pattern is observed in the two type strains of *Melaminivora* species (Kim et al., 2018). *Melaminivora jejuensis* strain KBB12^T utilizes a wider diversity of single carbon sources (polymers, sugars and derivatives thereof, organic acids) than *Melaminivora alkalimesophila* strain CY1^T (organic acids, amino acids). The *M. jejuensis* type strain harbors the gene *soxB*, an indicator of the presence of the Sox pathway and a putative indication of the ability of the organism to oxidize sulfur (Kim et al., 2018). In addition, *Melaminivora alkalimesophila* strain CY1^T is able to degrade melamine (1,3,5-triazine-2,4,6-triamine), a xenobiotic that belongs to the s-triazine family, widely used in laminates, plastics, and adhesives. Melamine degradation products are NH_3 and CO_2, formed under aerobic conditions (Wang et al., 2014a). When grown with 4.0 mM melamine as the single source of carbon, 94% of the xenobiotic was degraded after 10 days of incubation, with the transitory accumulation of ammeline, ammelide, cyanuric acid, biuret, and urea (Wang et al., 2014a).

7.5. Chemotaxonomic characteristics:

The major respiratory quinone is ubiquinone 8, and the major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. In addition, in *Melaminivora alkalimesophila* strain CY1^T an unidentified phospholipid and one unidentified aminophospholipid is described, whereas in *Melaminivora jejuensis* strain KBB12^T one phospholipid and one unidentified lipid is reported. According to Kim et al. (2018), the predominant fatty acids of the type strains of the two species of *Melaminivora* are summed feature 3 ($C_{16:1}$ *ω7c* / iso-$C_{15:0}$ 2-OH; 42.9% in both strains CY1^T and KBB12^T), $C_{16:0}$ (30.7 and 25.2% in strains CY1^T and KBB12^T, respectively), and $C_{18:1}$ *ω7c* (10.4 and 16.1% in strains CY1^T and KBB12^T, respectively). Other fatty acids include $C_{10:0}$ 3-OH (6.7 and 5.1% in strains CY1^T and KBB12^T, respectively) and $C_{12:0}$ (4.0 and 3.6% in strains CY1^T and KBB12^T, respectively).

7.6. Genome:

The draft genome of the *Melaminivora* alkalimesophila type strain CY1^T (=DSM 26006^T) is available under the DDBJ/EMBL/GenBank accession nos. NZ_ALEE00000000.1 (Wang et al., 2014b) and NZ_QGUB00000000.1 (DOE Joint Genome Institute, unpublished). The two sequences were obtained by different authors, using different technologies (Illumina GAII and Illumina HiSeq) and assemblers (Velvet and SPAdes), resulting in final draft genomes with some differences. The *M. alkalimesophila* genome has a size of 2.95-3.01 Mbp, with a total of 2525-2636 candidate protein-coding genes and a G+C content of 69.5-69.7 mol%.

7.7. Antibiotics susceptibility:

Melaminivora alkalimesophila strain CY1[T] is susceptible to cefatriaxone (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). *Melaminivora jejuensis* strain KBB12[T] is susceptible to ampicillin (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (30 µg), penicillin G (10 IU), polymyxin B (300 IU) and tetracycline (30 µg). It is not known which of these, if any, phenotypes are acquired or intrinsic.

7.8. Ecology:

Members of the genus *Melaminivora* were originally isolated from polluted habitats, namely wastewater sludge of a melamine-producing factory (*Melaminivora alkalimesophila*) and swinery waste (*Melaminivora jejuensis*).

Melaminivora jejuensis strain KBB12[T] was recovered from a bioreactor treating hydrocarbon-sulfide-containing wastewater, where both chemo-organo-heterotrophs of the genus *Acinetobacter* and chemo-litho-autotrophs, such as the sulfur oxidizers of the genera *Thiobacillus* and *Thiomonas* could survive (Liao et al., 2008). The presence of the gene *sox*B (accession number KC295221.1) in the genome of strain KBB12[T], which is part of the Sox pathway, may represent a biomarker of this kind of ecological niche (Meyer et al., 2007).

8. ENRICHMENT/ISOLATION PROCEDURES:

Melaminivora alkalimesophila strain CY1^T was isolated from an enrichment culture established from wastewater sludge of a melamine-producing factory in Sanming city, Fujian, China. Nitrate-free NMS medium (Yu et al., 2007), at pH 7.3–7.5, with melamine (500 mg L^{-1}) as the single carbon and nitrogen source was used in the enrichment. A volume of 100 mL of enrichment culture was incubated in a 250-mL sterile flask at 30 °C and 150 rpm, for one week. After that period, 50 mL of that culture was discarded and replenished with equal volume of fresh enrichment medium. This procedure was performed five times, after which the enrichment culture was spread onto R2A agar for the isolation of melamine-degrading organisms. Morphologically distinct colonies were sub-cultured on R2A agar and tested for the ability to degrade melamine (Wang et al., 2014a, Wang et al., 2014b).

Melaminivora jejuensis strain KBB12^T was recovered from an enrichment culture established from a sample collected at a waste site of a swinery field in Jeju, Republic of Korea. Medium BH, designed to recover autotrophic sulfur oxidizers, was used for the enrichment. Per liter it contains $MgSO_4.7H_2O$, 0.409 g; $CaCl_2.2H_2O$, 0.0265 g; KH_2PO_4, 1 g; NH_4NO_3, 1 g; $Na_2HPO_4 \cdot 12H_2O$, 6 g; $FeCl_3 \cdot 6H_2O$, 0.0833 g; 1 mL trace element solution, and $NaHCO_3$, 0.2 g; and $Na_2S_2O_3$, 4.0 g, as carbon and energy source, respectively. Strain KBB12^T was sub-cultivated on TSA at 30 °C (Kim et al., 2018).

9. MAINTENANCE PROCEDURES:

Recommended *Melaminivora* maintenance is on complex media such as R2A agar or TSA for short periods or in 20% (v/v) glycerol suspensions at -80 ºC for long-time preservation (Kim et al., 2018).

10. DIFFERENTIATION OF THE GENUS *MELAMINIVORA* FROM OTHER GENERA:

The nearest neighbor genera of *Melaminivora* are *Oryzisolibacter* (see gbm01828) and *Alicycliphilus* (see gbm01825). Differential characteristics between the type strains of these taxa are described in Table 1 of the genus *Oryzisolibacter* chapter (see gbm01828).

11. TAXONOMIC COMMENTS:

Based on the 16S rRNA gene sequence analysis, *Melaminivora* belongs to the family *Comamonadaceae.* The type strains of the two species of this genus described up to now share 97.2% 16S rRNA gene sequence similarity (Kim et al., 2018). The low DNA–DNA relatedness value (43.4 ± 2.7%) between *M. jejuensis* KBB12^{T} and *M. alkalimesophila* DSM 26006^{T} (=CY1^{T}), which is below the 70% cut-off value recommended for the assignment of a strain to the same species (Wayne et al., 1987), supported the separation of these organisms into distinct species. In addition, *M. jejuensis* KBB12^{T} and *M. alkalimesophila* DSM 26006^{T} can be differentiated through phenotypic characteristics (Table 1).

Oryzisolibacter propanilivorax strain EPL6^{T} is the nearest neighbour species of *Melaminivora*, sharing 96.8% and 96.7% 16S rRNA gene sequence identity with the type strains of *M. alkalimesophila* and *M. jejuensis*, respectively. Lower values (95.5-95.7%) are shared between the *Melaminivora* type strains and *Alicycliphilus denitrificans* strain K601^{T}, and with the members of the genera *Diaphorobacter*, *Acidovorax* and *Comamonas* (< 95.5%).

<Table 1 near here>

12. LIST OF SPECIES OF THE GENUS *MELAMINIVORA*:

1. ***Melaminivora alkalimesophila*** Wang, Li, Hu, Qin, Xu and Yu, 2014, 1943[VP]

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* (from Gr. adj. *philos*) friend, loving; N.L. fem. adj. *alkalimesophila*, loving alkaline and mesophilic conditions].

In addition to the characteristics given in the genus description and Table 1, this organism shows the following properties: tests positive for indole and assimilates L-alanyl glycine, L-leucine, L-pyroglutamic acid, methyl pyruvate, monomethyl succinate, propionic acid, and β-hydroxybutyric acid; does not assimilate α-cyclodextrin, 2,3-butanediol, 2-aminoethanol, acetic 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, DL-α-glycerol phosphate, D-mannitol, 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, phenylethylamine, putrescine, quinic acid, sucrose, thymidine, turanose, 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. Tests negative for D-glucose fermentation, and the utilization of capric acid, D-mannose, malic acid, potassium gluconate and trisodium citrate as a carbon source.

Produces esterase (C4), leucine aminopeptidase, naphthol-AS-BI-phosphoamidase and valine aminopeptidase, acid phosphatase, alkaline phosphatase, cystine aminopeptidase, esterase lipase (C8) and lipase (C14); but does not produce N-acetyl-β-glucosaminidase, trypsin, α-chymotrypsin, α-fucosidase, α-galactosidase, α-glucosidase, α-mannosidase, β-galactosidase, β-glucosidase and β-glucuronidase. Antibiotic susceptibility profile includes resistance to antibiotics belonging to different classes.

The DNA G+C content (mol %) is 69.5 (HPLC).

Type strain: CY1 (=CCTCC AB 2012024 = DSM 26006)

GenBank accession number (16S rRNA): JQ676982

GenBank accession number (genome): ALEE00000000.1 and NZ_QGUB00000000.1

2. ***Melaminivora jejuensis*** Kim, Park, Lee, Song, Kim, 2018, 11^{VP}

jejuensis [je.ju.en'sis. N.L. fem. adj. *jejuensis*, pertaining to Jeju, Republic of Korea, from where the type strain was isolated].

In addition to the genus description and Table 1, this organism shows the following traits: utilizes N-acetyl mannosamine, D-arabitol, glucuronamide, gluconate, lactulose, malate, palatinose, raffinose, D-sorbitol, β-hydroxybutyric acid, and malic acid as single carbon source.

Produces acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase and naphthol-AS-BI-phosphohydrolase, but not lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine decarboxylase or ornithine decarboxylase. Does not hydrolyse Tween 60, casein, urea, DNA, tyrosine, aesculin, cellulose and starch. Shows susceptibility to antibiotics belonging to different classes.

The DNA G+C content (mol %) is 69.6 (HPLC).

Type strain: KBB12 (=KCTC 32230 =JCM 18740)

GenBank accession number (16S rRNA): JX997988

RELATED ARTICLES:

gbm01825

gbm01828

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TABLES:

Table 1. Differentiating characteristics of the type strains of the validly named species of the genus *Melaminivora*.

Characteristic	***Melaminivora alkalimesophila*** **DSM 26006^T**	***Melaminivora jejuensis*** **KBB12^T**
Colony colour	Translucent	Pale yellow
Cell morphology	Rods (0.7–0.9 µm x 2.0–3.0 µm)	Rods (0.5–0.7 µm x 2.0–3.5 µm)
Optimal growth temperature (°C)	40–45	30–37
Optimal growth pH	9.5	7-8
NaCl tolerance range (%, w/v)	0–7	0-1
Assimilation of:		
N-Acetyl-D-galactosamine	-	+
N-Acetyl-D-glucosamine	-	+
Adipate	-	+
Adonitol	-	+
L-Alanine	+	-
L-Asparagine	+	-
Bromosuccinic acid	-	+
D-Glucuronic acid	-	+
L-Glutamic acid	+	-
L-Lactic acid	+	-
Maltose	-	+
D-Mannose	-	+
myo-Insitol	-	+
p-Hydroxy-phenylacetic acid	-	+

Phenylacetate	-	+
L-Phenylalanine	+	-
L-Proline	+	-
L-Pyroglutamic acid	+	-
Sebacic acid	-	+
Succinamic acid	+	-
Succinic acid	-	+
Succinic acid mono-methyl ester	+	-
Sucrose	-	+
Activity of:		
Arginine dihydrolase	+	-
Urease	+	-
Hydrolysis:		
Gelatin	-	+
Tween 20; Tween 40; Tween 80	-	+
Polar lipids	PE, PG, DPG, APL, PL	PE, PG, DPG, PL, UL

PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; APL, unidentified aminophospholipid; PL, unidentified phospholipid; UL, unidentified lipid.

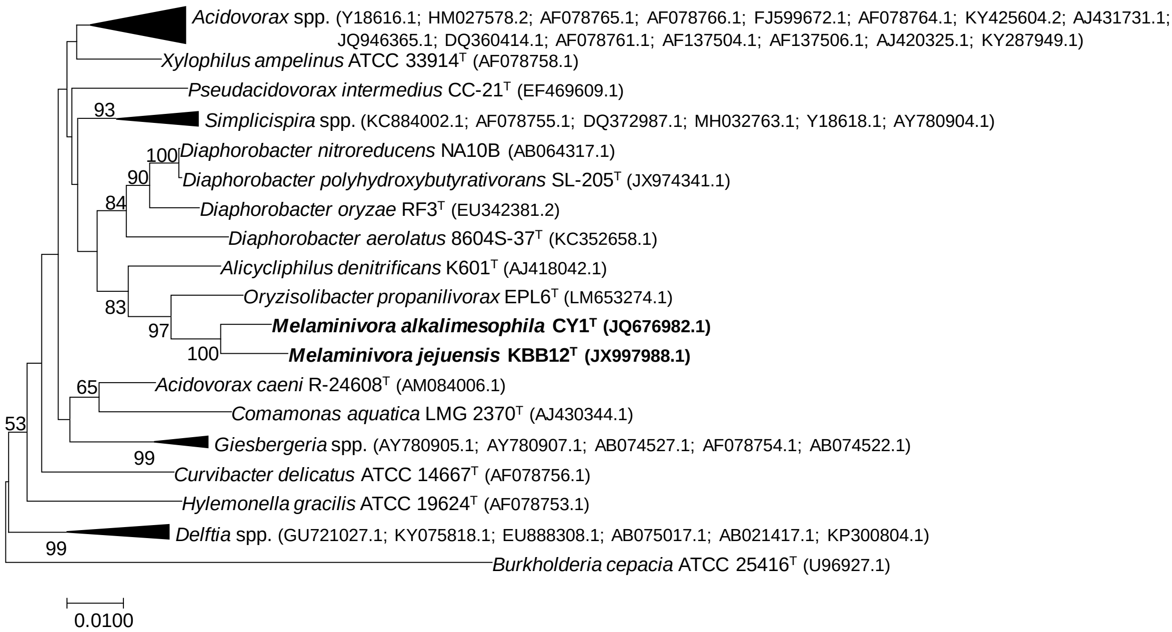

Figure 1. Phylogenetic tree based on the 16S rRNA gene sequence showing the position of the genus *Melaminivora* within the family *Comamonadaceae*. Clusters represent species of monophyletic genera. The phylogenetic tree was inferred using the Neighbor-Joining method (Saitou and Nei, 1987) in MEGA7 (Kumar et al., 2016). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The analysis involved 47 nucleotide sequences and a total of 1304 positions. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Bar: 1 nucleotide substitution per 100 nucleotide positions. *Burkholderia cepacia* was used as outgroup.