Emended description of the genus *Methylophaga* Janvier *et al.* 1985

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The genus *Methylophaga* Janvier *et al.* 1985 comprises eight species with validly published names at the time of writing. The original description of the genus was published over 26 years ago and was based on only two species, namely *Methylophaga marina* and *Methylophaga thalassica* – as such, the description of the genus requires updating to take into account the other six known species. Based on literature concerning the eight species of *Methylophaga* published over the last 26 years, an emended description of the genus is presented, taking into account properties of all members of the species with validly published names.

The genus *Methylophaga* was designated in 1985 following the isolation of a novel species of halophilic marine methylotrophic gammaproteobacteria, named *Methylophaga marina*, and the reclassification of '*Methylomonas thalassica*' as *Methylophaga thalassica* (Janvier *et al.*, 1985). The description of the genus was based on a number of strains of the only two species then known, and indicates that all members of the genus are 'auxotrophic for B₁₂' and 'isolated from marine environments'. Following the description to date of a further six *Methylophaga* species with validly published names, namely *M. alcalica* (Doronina *et al.*, 2003a), *M. aminisulfidivorans* (Kim *et al.*, 2007), *M. lonarensis* (Antony *et al.*, 2012), *M. muralis* (Doronina *et al.*, 2005, 2011), *M. sulfidovorans* (de Zwart *et al.*, 1996, 1998) and *M. thiooxydans* (Boden *et al.*, 2010, 2011), along with the isolation of further strains of *M. marina* that do not require vitamin B₁₂ (Li *et al.*, 2007), it is clear that the description of the genus requires updating to bring it in line with present knowledge of the genus. A number of other strains have been designated species of the genus *Methylophaga*, namely '*M. limanica*' (Doronina *et al.*, 1997) and '*M. natronica*' (also known as '*M. buryatica*'; Doronina *et al.*, 2003b), although these names have not been validly published and the strains are therefore not included in the updated description. Genome sequences are now available for *M. aminisulfidivorans* (Han *et al.*, 2011) and *M. thiooxydans* (Boden *et al.*, 2011), and will assist in further characterization of members of this genus.

The phylogenetic positions, with respect to 16S rRNA gene sequences, of the eight *Methylophaga* species with validly published names are shown in Fig. 1. It can be seen that the three alkaliphilic species, *M. alcalica*, *M. lonarensis* and *M. muralis*, form a distinct clade away from that containing the type species (*M. marina*) – this is consistent with the relatively low identities of these sequences to that of the type species, as shown in Table 1. Additionally, the DNA G+C contents of *M. alcalica* and *M. lonarensis* are the

highest within the genus (see Table 1), although that of *M. muralis* is more similar to those of non-alkaliphilic species. Further studies into the chemotaxonomic properties of the three alkaliphilic *Methylophaga* species, along with DNA–DNA hybridization against *M. marina*, are required to verify their position, although, for the sake of this emendation, they are included in the genus *Methylophaga*.

Differential physiological and chemotaxonomic properties are given in Table 2. It can be seen that the predominant cellular fatty acids have only been determined in half of the species, although palmitic (C_{16:0}) and palmitoleic (C_{16:1}) acids are found in all species analysed and vaccenic acid (C_{18:1ω7c}) predominates in all of the alkaliphilic species; it is also present at low levels (2.3 %) in *M. aminisulfidivorans* (Kim *et al.*, 2007). Dominant cellular phospholipids are phosphatidylethanolamine and phosphatidylglycerol in those species analysed (*M. alcalica*, *M. aminisulfidivorans*, *M. lonarensis* and *M. muralis*; references in Table 2). Given that these parameters have not been determined for the type species, they are not included in the emended description, but are included here for completeness. In terms of substrate ranges, *M. lonarensis* appears to be the most restricted, growing only on methanol, which is a substrate common to all members of the genus, although the majority also grow on monomethylamine and fructose (as shown in Table 2). *M. aminisulfidivorans*, *M. sulfidovorans* and *M. thiooxydans* also grow on dimethylsulfide, with the latter two species exhibiting chemolithoheterotrophy at the expense of sulfide and thiosulfate, respectively (de Zwart *et al.*, 1996; Boden *et al.*, 2010). Fructose is the most commonly used multicarbon compound, although substituted thiophenes and higher alkanesulfonates can be used by some species, albeit slowly (Boden *et al.*, 2011).

It is clear that many species have properties that conflict with the description as given by Janvier *et al.* (1985)

Fig. 1. Maximum-likelihood tree showing the phylogenetic positions of members of the genus *Methylophaga* based on 1366 nt of the 16S rRNA gene sequences, using the Tamura–Nei model. Bootstrap values (%) are given from 500 replicates. Bar, 1 difference per hundred. GenBank accession numbers are given in parentheses.

– namely with respect to B₁₂ auxotrophy, alkaliphilicity and physiological properties. To rectify conflicts and to bring the description into line with information about *Methylophaga* species gained over the last 26 years, the following emended description of the genus is proposed, taking into account the properties of all eight species with validly published names to date.

Table 1. Differential molecular properties of type strains of the eight currently recognized species of the genus *Methylophaga*

<i>Methylophaga</i> species	Type strain	DNA G + C content (mol%)	16S rRNA gene identity (%) to <i>M. marina</i>	Reference
<i>M. alcalica</i>	M39 ^T	48.3	94	Doronina <i>et al.</i> (2003a)
<i>M. aminisulfidivorans</i>	MP ^T	44.9	96	Kim <i>et al.</i> (2007)
<i>M. lonarensis</i>	MPL ^T	50.0	94	Antony <i>et al.</i> (2012)
<i>M. marina</i>	222 ^T	43.0	100	Janvier <i>et al.</i> (1985)
<i>M. muralis</i> *	Kr3 ^T	44.6	93	Doronina <i>et al.</i> (2005)
<i>M. sulfidovorans</i>	RB-1 ^T	42.4	97	de Zwart <i>et al.</i> (1996)
<i>M. thalassica</i> †	ATCC 34146 ^T	44.0	97	Janvier <i>et al.</i> (1985)
<i>M. thiooxydans</i> ‡	DMS010 ^T	45.9	95	Boden <i>et al.</i> (2010)

*Originally named '*Methylophaga murata*'.

†Originally named '*Methylomonas thalassica*'.

‡Originally named '*Methylophaga thiooxydans*'.

Table 2. Differential physiological and chemotaxonomic properties of species of the genus *Methylophaga*

Species: 1, *M. alcalica* (Doronina *et al.*, 2003a); 2, *M. aminisulfidivorans* (Kim *et al.*, 2007); 3, *M. lonarensis* (Antony *et al.*, 2012); 4, *M. marina* (Janvier *et al.*, 1985); 5, *M. muralis* (Doronina *et al.*, 2005); 6, *M. sulfidovorans* (de Zwart *et al.*, 1996); 7, *M. thalassica* (Janvier *et al.*, 1985); 8, *M. thiooxydans* (Boden *et al.*, 2010). ND, Parameters not determined by the original authors; MMA, monomethylamine.

Property	1	2	3	4	5	6	7	8
Type strain	M39 ^T	MP ^T	MPL ^T	222 ^T	Kr3 ^T	RB-1 ^T	ATCC 34146 ^T	DMS010 ^T
Growth temperature (°C)	4–35	ND	20–37	10–40	0–42	ND	10–40	ND
Growth pH	8–11	6–8	7–10	ND	6–11	ND	ND	ND
Vitamin B ₁₂ auxotrophy	+	–	–	+	+	+	+	–
Predominant cellular fatty acids	C _{16:0} ; C _{16:1} ; C _{18:1}	C _{16:0} ; C _{16:1}	C _{16:0} ; C _{16:1} ; C _{18:1}	ND	C _{16:0} ; C _{16:1} ; C _{18:1}	ND	ND	ND
Growth on fructose	–	+	–	+	+	–	+	+
Growth on MMA	+	+	–	+	+	+	+	+
Source	Saline soda lake	Seawater	Soda lake	Seawater	Marble wall	Marine microbial mat	Seawater	Algal culture

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This emended description is based on that of Janvier *et al.* (1985) with emendations based on de Zwart *et al.* (1996), Doronina *et al.* (2003a, 2005), Li *et al.* (2007), Kim *et al.* (2007), Boden *et al.* (2010) and Antony *et al.* (2012).

Cells are non-sporulating, Gram-negative rods, motile by means of a single polar flagellum. Thick periplasmic space of 20–30 nm. Strictly aerobic moderate halophile. Some species are alkaliphilic. Some species reduce nitrate to nitrite. Some strains are auxotrophic for vitamin B₁₂. The dominant respiratory quinone is UQ-8. Does not grow on complex media. Does not grow in the absence of NaCl. Catalase- and oxidase-positive. One-carbon compounds are used as sole carbon and energy sources by all strains, with carbon assimilation proceeding via the KDPG-variant RuMP pathway. Does not grow on methane. Some species grow on a limited range of multicarbon compounds, namely fructose, substituted thiophenes or long-chain alkanesulfonates. Chemolithoheterotrophy is observed in some species. Autotrophic growth is not observed. DNA G+C contents range from 42 to 50 mol%. Typically isolated from environments of low water activity, such as marine environments.

The type species is *Methylophaga marina*. Other species with validly published names are *M. alcalica*, *M. aminisulfidovorans*, *M. lonarensis*, *M. muralis*, *M. sulfidovorans*, *M. thalassica* and *M. thiooxydans*.

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