Proposal of *Oscillochloridaceae* fam. nov. on the basis of a phylogenetic analysis of the filamentous anoxygenic phototrophic bacteria, and emended description of *Oscillochloris* and *Oscillochloris trichoides* in comparison with further new isolates

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The nucleotide sequences of the genes of 16S rRNAs were determined for the type strain *Oscillochloris trichoides* DG-6^T and three new strains of *Oscillochloris*-like mesophilic filamentous green bacteria. Two major clusters have been found within the family *Chloroflexaceae* by phylogenetic analysis: one cluster includes thermophilic species of *Chloroflexus* and the second includes mesophilic strains of *Oscillochloris*. The degree of relatedness of these clusters was below an intergeneric level, having only 82.5–86.5% of 16S rDNA sequence similarity. These phylogenetic data correlate well with the significant physiological, biochemical and chemotaxonomical differences between members of both groups. Therefore, the *Oscillochloris* and *Chloroflexus* clusters should be considered as two separate families. The description of the new family, *Oscillochloridaceae* fam. nov., and emended descriptions of the genus *Oscillochloris* and the species *Oscillochloris trichoides* are presented.

Keywords: filamentous green bacteria, *Oscillochloridaceae* fam. nov., *Oscillochloris trichoides*, phylogenetic analysis, taxonomic revision

INTRODUCTION

The thermophilic filamentous green bacterium *Chloroflexus aurantiacus* (Pierson & Castenholz, 1974) was the first-discovered genus and species of the family *Chloroflexaceae*. The new family *Chloroflexaceae* was proposed by Trüper (1976), on the basis of the properties of *Chloroflexus aurantiacus*, and placed in the suborder *Chlorobiales*. Trüper based his classification on a set of bacteriochlorophylls (*c* and *a*) and special antenna structures (chlorosomes). These properties, found in both *C. aurantiacus* and members of *Chlorobiaceae*, distinguished them from the purple phototrophic bacteria and the subsequently described

heliobacteria. However, the bacteria consequently ascribed to the new family *Chloroflexaceae* showed substantial morphological and physiological differences. They form multicellular trichomes, have gliding motility and have flexible cell walls. They grow best as photoheterotrophs and are able to grow aerobically in darkness, they have a unique CO₂-fixation pathway and they are incapable of nitrogen fixation, etc. Furthermore, the new family differed significantly from the previously known unicellular green sulfur bacteria of the family *Chlorobiaceae* (Pierson & Castenholz, 1974, 1995). On the basis of a 16S rRNA analysis, it was concluded that as all green sulfur bacteria and *Chloroflexus*-like organisms formed separate deep eubacterial lines of descent (Gibson *et al.*, 1985; Woese, 1987; Stackebrandt *et al.*, 1996), they could not belong to the same suborder.

All previous taxonomic descriptions of the family *Chloroflexaceae* have been based on the study of *C.*

This paper is dedicated to the memory of Professor Elena N. Kondratieva (1925–1995).

The GenBank accession numbers of the 16S rDNA sequences of *Oscillochloris trichoides* strain DG-6^T and *Oscillochloris* strains BM, A-19 and R are AF093427, AF149018, AF146831 and AF146832, respectively.

aurantiacus, the only species that had been successfully isolated and sustained in pure culture. However, additional filamentous phototrophic bacteria are now known. Pfennig (1989) grouped them as 'multicellular filamentous green bacteria' including the genera *Chloroflexus*, *Chloronema*, *Oscillochloris* and *Heliothrix*. Pierson & Castenholz (1995) correctly noted that this name was not accurate, as *Heliothrix*, apparently different from the other species of the group, has only bacteriochlorophyll *a* (not *c*, *d* or *e*) and does not contain chlorosomes. These authors proposed that the group should be named 'anoxygenic filamentous phototrophic bacteria'. It should be mentioned that it is a very diverse group that comprises photoautotrophic and photoheterotrophic organisms with or without chlorosomes, facultative anaerobes and strict anaerobes, thermophilic and mesophilic bacteria and salt- and freshwater forms.

Most of the proposed 'species' have been studied as natural populations or in binary or mixed cultures (Gorlenko & Korotkov, 1979; Pierson *et al.*, 1994). Mesophilic freshwater strains of *Chloroflexus*-like bacteria isolated earlier (Gorlenko, 1975) were lost. Until recently, pure cultures were available only for some strains of *C. aurantiacus*. The description of the second thermophilic species of this genus, *Chloroflexus aggregans* (Hanada *et al.*, 1995), did not lead to any essential changes in the characteristics of the family *Chloroflexaceae*. Therefore the taxonomy and phylogenetic position of filamentous anoxygenic phototrophic bacteria remains unclear. Consequently, broadly based phenotypic data and nucleic acid sequences are needed to construct useful taxa and meaningful phylogenies.

Research on the first pure culture of the mesophilic filamentous green bacterium *Oscillochloris trichoides* has been essential in expanding our knowledge of this bacterial group (Gorlenko & Pivovarova, 1977; Gorlenko & Korotkov, 1979; Keppen *et al.*, 1989, 1993, 1994; Ivanovsky, 1993; Ivanovsky *et al.*, 1999). The photosynthetic apparatus of *O. trichoides* DG-6^T as well as that of *C. aurantiacus* contains chlorosomes with bacteriochlorophylls *c* and α -, β - and γ -carotenes as light-harvesting pigments. The *in vivo* absorption spectra of cell suspensions of *O. trichoides* DG-6^T exhibited maxima at 456, 748 and 852 nm. In contrast to *C. aurantiacus*, *O. trichoides* strain DG-6^T performed autotrophic CO₂ fixation using the reductive pentose phosphate cycle (the Calvin cycle), with ribulose 1,5-bisphosphate carboxylase and phosphoribulokinase as the key enzymes (Ivanovsky, 1993; Ivanovsky *et al.*, 1999). The reductive tricarboxylic acid cycle typical of the green sulfur bacteria (Evans *et al.*, 1966) was not found in *O. trichoides*. The 3-hydroxypropionate cycle (Strauss & Fuchs, 1993) or reductive dicarboxylic acid cycle (Ivanovsky *et al.*, 1993), proposed for *C. aurantiacus* strains OK-70fl and B-3, respectively, was not found in *O. trichoides*. Unlike *C. aurantiacus* (Krasilnikova *et al.*, 1986), *O. trichoides* has a functionally incomplete tricarboxylic

acid cycle lacking 2-oxoglutarate dehydrogenase and it lacks the glyoxylate bypass (Keppen & Krasilnikova, 1995; Ivanovsky *et al.*, 1999). *O. trichoides* DG-6^T can use only a limited number of organic compounds (acetate, pyruvate) for photolithoheterotrophic growth, whereas it is unable to grow as a chemoheterotroph under aerobic or microaerobic conditions in the dark (Keppen *et al.*, 1994). DNA-DNA hybridization experiments indicate the existence of remote genetic relationships between *O. trichoides* DG-6^T and *C. aurantiacus* (less than 5% homology). 5S rRNA sequence analysis supported the fact that they form a monophyletic group (Keppen *et al.*, 1994). Thus, phenotypic and genotypic differences between species of the genera *Chloroflexus* and *Oscillochloris* were substantial enough to allow the proposal that *Oscillochloris* represents an independent family.

To establish the phylogenetic position of the genus *Oscillochloris*, we have undertaken a sequence analysis of 16S rDNA genes of strain DG-6^T of *O. trichoides* and three new strains of *Oscillochloris* spp. which had been isolated from different environments. On the basis of the phylogenetic data and the unique physiological properties of the studied strains of *Oscillochloris*, we propose the family *Oscillochloridaceae* fam. nov.

METHODS

Strains and growth conditions. Four strains of mesophilic filamentous green bacteria were isolated and studied in pure culture. *O. trichoides* strain DG-6^T was isolated from microbial mats of warm hydrogen sulfide springs in the Caucasus region of SE Europe (Keppen *et al.*, 1993, 1994). Strain R was isolated from shallow river waters near Moscow (the Moscow River), strain BM was from a littoral estuary of the White Sea (Nilma Guba) and strain A-19 was from an alkaline soda lake (Lake Gonzogor; pH 9.3, total dissolved salts 0.9 g l⁻¹) in SE Siberia. The thermophilic filamentous green bacterium *C. aurantiacus* (strain OK-70fl) was used for comparative studies.

All strains were maintained on modified medium DGN (Castenholz & Pierson, 1981; Keppen *et al.*, 1994). For strain A-19, the pH was adjusted to 9.0 by the addition of NaHCO₃ (2.5 g l⁻¹) and Na₂CO₃ (2.5 g l⁻¹). The organisms were routinely cultivated at 1000 lx in 100 ml screw-cap bottles completely filled with the medium. The inoculum was 10–15% (v/v) and the bottles were incubated at 28 °C for mesophilic species and at 55 °C for *C. aurantiacus*.

Fatty acids and quinone composition. The fatty acids were analysed as described previously (Zhilina *et al.*, 1997), using a Hewlett Packard HP-5973 mass spectrometer. The quinones were assayed according to Collins (1985), using a Finnigan MAT 8430 mass spectrometer.

16S rRNA sequencing and analysis. The 16S rRNA gene was selectively amplified from genomic DNA by the PCR, using 5'-AGAGTTTGATCCTGGCTCAG-3' as the forward primer and 5'-TACGTTTACCTTGTTACGACTT-3' as the reverse one (Lane, 1991). The PCR was performed in 100 µl reaction mixture, which consisted of 1 µg DNA template, 200 µM (each) primers, 200 µM (each) deoxynucleoside triphosphates and 3 U Tet-z polymerase

(BioMaster) in reaction buffer (100 mM Tris/HCl, 500 mM KCl, 20 mM MgCl₂; pH 8.3). The temperature cycling was done in 30 amplification cycles: 1 min at 94 °C, 1 min at 42 °C and 1 min at 72 °C. The final extension was performed at 72 °C for 6 min. The PCR products were purified with the PCR prep kit (Promega), as recommended by the manufacturer. The 16S rRNA gene was sequenced in both directions with the forward and reverse primers. DNA sequencing was performed with Sequenase version 2 from USB. The 16S rDNA sequence was aligned with the sets of 16S rRNA sequences, which had been obtained from either the most recent versions of the Ribosomal Database Project or from GenBank, by using MULTALIN software (Corpet, 1988). Phylogenetic dendrograms were constructed by treeing algorithms from the GENEBEE package (Brodsky *et al.*, 1991), the PHYLIP package (Felsenstein, 1989) and the TREECON package (Van de Peer & De Wachter, 1994).

Nucleotide sequence accession numbers. The accession numbers of the sequences used as references were as follows: *C. aurantiacus* J-10-fl^T, M34116; *C. aurantiacus* Y400-fl, L05508, L05509, L05510, L04674; *C. aggregans* MD-66^T, D32255; *Herpetosiphon aurantiacus* ATCC 23779^T, M34117; *Herpetosiphon* sp. F018, X86447; *Herpetosiphon geysericola* ATCC 23076^T, AF039293; *Heliothrix oregonensis* F1^T, L04675; *Thermomicrobium roseum* ATCC 27502^T, M34115; *Sphaerobacter thermophilus* S 6022^T, X53210; *Dehalococcoides ethenogenes* 195^T, AF004928; *Thermus aquaticus* YT-1^T, L09663; *Meiothermus ruber* ATCC 35948^T, Z15059; *Deinococcus radiodurans* ATCC 35073^T, M21413; *Chlorobium limicola* 8327, M31769.

RESULTS AND DISCUSSION

Characteristics of *Oscillochloris* strains

All of the strains of mesophilic filamentous green bacteria studied were grown in the light under anaerobic conditions only. Strains R, BM and A-19 were morphologically similar to *O. trichoides* DG-6^T (Keppen *et al.*, 1994). They all had green, flexible, gliding, non-ramified multicellular filaments, 1.0–1.5 µm in diameter, with or without sheaths. The lengths of the individual cells in filaments varied from 2.0 to 5.0 µm. The cells usually contained gas vesicles, which were localized along the cell septa. The bacteria deposited poly-β-hydroxybutyrate inside the cells. Globules and crystals of sulfur were deposited outside the cells in the immediate proximity of the trichomes. Cell suspensions were dark green. Chlorosomes served as light-harvesting structures of the photosynthetic apparatus. Bacteriochlorophyll *c* was the major photosynthetic pigment. A small amount of bacteriochlorophyll *a* was also present.

The preferred mode of growth was photolithoheterotrophic, but strains were also able to grow photoautotrophically with sulfide as an electron donor and CO₂ as the sole carbon source. All of these strains oxidized sulfide only to elemental sulfur. They were mesophilic, with optimal growth at 28–30 °C. Strains exhibited growth at pH 6.8–9.0, with the optimum at pH 7.5–8.5. The optimal concentration of sulfide (Na₂S.9H₂O) was 0.5–0.7 g l⁻¹. Sulfide was tolerated at levels up to 1.5 g l⁻¹.

Table 1. Fatty acid composition of *Oscillochloris trichoides* DG-6^T and *Chloroflexus aurantiacus* OK-70fl

	Fatty acid	DG-6 ^T	OK-70fl
1.	14:0	0.31	0.12
2.	15:0	0.23	1.08
3.	15alk	0.25	0.06
4.	16:1	15.93	1.01
5.	16:0	30.33	19.23
6.	16alk	9.65	1.56
7.	h16	0.61	0.00
8.	17:1	0.23	1.43
9.	17:0	0.42	7.75
10.	17alk	0.00	0.89
11.	18:1	38.28	13.86
12.	18:0	2.74	30.61
13.	18alk	1.02	9.63
14.	19:1	0.00	1.93
15.	19:0	0.00	6.31
16.	19alk	0.00	1.68
17.	20:1	0.00	1.65
18.	20:0	0.00	1.20

Strains BM and R, as well as *O. trichoides* DG-6^T, assimilate CO₂ via the Calvin cycle. They lack a complete TCA cycle and a glyoxylate bypass, can use only a limited number of organic compounds (acetate, pyruvate) for photolithoheterotrophic growth and are unable to grow as chemoheterotrophs under aerobic or microaerobic conditions in the dark.

Fatty acids and quinone composition

The fatty acid composition of *O. trichoides* DG-6^T and *C. aurantiacus* OK-70fl, as well as that of other phototrophic bacteria, was characterized by the predominance of C-16 and C-18 fatty acids (Table 1). However, detailed study revealed significant qualitative and quantitative differences in fatty acid composition between *O. trichoides* DG-6^T and *C. aurantiacus* OK-70fl. Unlike *C. aurantiacus* OK-70fl, *O. trichoides* DG-6^T contained an 18:1 (38 %) component with a large amount of C-16 (56 %), whereas *C. aurantiacus* OK-70fl contained C-18 fatty acids in higher proportions (54 %) than C-16 fatty acids (22 %). The relative concentrations of 17:1 and 17:0 in *O. trichoides* were much lower than in *C. aurantiacus*, and C:17alk was not detected. In addition, *O. trichoides* DG-6^T was unable to synthesize C-19 and C-20 fatty acids. The ratio of saturated to unsaturated fatty acid in *O. trichoides* DG-6^T (0.84), as compared to that of phototrophic purple and green sulfur bacteria (0.9–1.2), revealed significant differences as compared with thermophilic *Chloroflexaceae* species (3.6–3.8) and with *Heliobacterium* and *Heliobacillus* (0.2–0.3) (Imhoff, 1988; Imhoff & Bias-Imhoff, 1995).

On the whole, the comparison of our fatty acid composition data with the results obtained for other

Fig. 1. Phylogenetic dendrogram of the phylum 'green non-sulfur bacteria and their relatives' based on the results of rDNA sequence comparisons. Pairwise similarity values were corrected according to Jukes & Cantor (1969) and used to reconstruct a neighbour-joining tree (Saitou & Nei, 1987). Bootstrap values (expressed as percentages of 100 replications) are shown at branch points; values greater than 95 were considered significant. Bar represents 5 nucleotide substitutions per 100 nucleotides.

phototrophic bacteria (Imhoff, 1988; Imhoff & Bias-Imhoff, 1995) shows that the fatty acid composition of *O. trichoides*, while different from that of heliobacteria, members of the *Chlorobiaceae* or thermophilic members of the *Chloroflexaceae*, is similar to that of some purple bacteria.

Menaquinone (MK-10) was the only quinone found in both *O. trichoides* DG-6^T and *C. aurantiacus*.

Analysis of 16S rDNA sequence data

The almost complete 16S rDNA sequences (nearly 1400 nucleotides long) were determined for the strain *O. trichoides* DG-6^T and for three new isolates (BM, A-19 and R). The G+C contents of these sequences were 60.4–61.3 mol %. On the basis of the phenotypic analysis and the 5S rRNA sequences (Keppen *et al.*, 1994), *O. trichoides* belongs to the eubacterial line of descent, formerly known as the 'green non-sulfur bacteria and their relatives', which lacked adequate phenotypic justification (Woese, 1987). This phylum contains two thermophilic green filamentous gliding phototrophs, *C. aurantiacus* and *C. aggregans*, the filamentous gliding non-photosynthetic bacterium *H. aurantiacus* and the unicellular non-photosynthetic thermophilic species *T. roseum* and *S. thermophilus* (Stackebrandt *et al.*, 1996).

Recently, a new bacterium, *Dehalococcoides ethenogenes*, was described which reductively dechlorinates tetrachloroethene to ethane. This bacterium may also be distantly related to the phylum (Maymo-Gatell *et al.*, 1997). Some micro-organisms, previously studied as natural populations, also belong to this phylum. These include a thermophilic filamentous gliding phototroph, *H. oregonensis*, which, unlike *Chloroflexus* and *Oscillochloris*, lacks chlorosomes and bacteriochlorophylls *c*, *d* or *e* (Weller *et al.*, 1992), as well as certain environmental clones of uncultured eubacteria (Weller *et al.*, 1991, 1992; Ward *et al.*, 1992; Giovannoni *et al.*, 1996; Ferris *et al.*, 1996). The *Deinococcus*–*Thermus* line of descent is the one most closely related to this phylum.

The lineage of the 'green non-sulfur bacteria' is dominated by thermophilic species. The G+C con-

tents of the rRNAs, like that in other thermophilic prokaryotes (Achenbach-Richter *et al.*, 1987; Murzina *et al.*, 1988), tend to range from 58 to 66 mol %, while typical mesophilic bacterial rRNAs usually have G+C contents of approximately 55%. Despite the fact that the *Oscillochloris* strains are mesophilic, the G+C contents of their 16S rDNA sequences fell into the range typical for the phylum, i.e. 60.4–61.3 mol %. Thus, a phylogenetic tree inferred from a transversion analysis (Swofford & Olsen, 1990; Woese *et al.*, 1991), does not contradict the inferred normal analysis, excluding the instability of the *D. ethenogenes* (51.9 mol % G+C in the 16S rDNA sequence) branching point (data not shown). It can be suggested that the members of the genus *Oscillochloris* had thermophilic ancestors that adapted to temperate or even cold (White Sea) habitats.

For many organisms of the phylum 'green non-sulfur bacteria and their relatives' only partial sequences of 16S rRNA are known. For phylogenetic analysis, therefore, we used several sets of sequences of 16S rDNA containing the most complete sequences (more than 1400 nucleotides) and different parts of the sequences. Sequence analysis included a 'maximum topological similarity' algorithm (Chumakov & Yushmanov, 1988), neighbour-joining (Saitou & Nei, 1987) and the maximum-parsimony analysis and distance matrix algorithm of De Soete (1983). All methods produced very similar patterns of relatedness and tree topologies.

On the phylogenetic tree (Fig. 1), the *Oscillochloris* strains clearly belong to the phylum of 'green non-sulfur bacteria and their relatives' (69.2–86.5 % sequence similarity) with a high level of bootstrap probability. This is supported by the presence of all of the distinctive 'signatures' and 16S rRNA structural idiosyncrasies specific to this phylum (Woese, 1987). The new strains (R, BM and A-19) form a distinct genomic group (97.0–98.5 % sequence similarity). The sequence similarity between the new strains of *Oscillochloris* and *O. trichoides* strain DG-6^T was 93.9–94.4 %. Taking into account the low level of sequence similarity between the new strains and *O. trichoides* DG-6^T, they may be described in future as new species of

Table 2. Sequence 'signatures' of 16S rRNA-differentiated *Oscillochloris*, *Chloroflexus* and other members of the phylum 'green non-sulfur bacteria and relatives'

Base pairing (:) was deduced from secondary-structure assignment. Lower-case letters indicate bases found in less than 50 % of assayable cases.

Sequence position*	Corresponding bases		
	<i>Oscillochloris</i> spp.	<i>Chloroflexus</i> spp.	Common to other members of phylum†
131:231	U:A	C:G	C:G
138:225	U:A	C:G	C(g):G(c)
139:224	C:G	G:C(u)	G,U:U(c,g)
140:223	A:U	G:C	G(a):C(u)
152	A	C	A(g)
154:167	U(g):A	C:G	G(c,u):C(g,a)
157:164	G:C	U:A	G,U(c):U,A(g)
166	A	G	U(g,a)
169	C	A	C(g)
291:309	U:A	C:G	U:A
412	U	C	U(g)
443:491	U:G	G:C	G(c):U(g)
493	C	A	C(g)
514:537	C:G	U:A	C:G
560	C	G	A,G(u)
579:762	A:U	G:C	G:C
592:647	U:A	G:U(c)	A(c,g,u):U(g,c)
615:625	A:U	C:G	C,G(a):G,C(u)
668:738	A(g):U	G:C	A(c):U(g)
686	U	G	U
721	U	G(a)	A(g)
811	U	C	A(g,c)
824:877	A:U	G:C	U(g,c):A(c,g)
831	U	C	U
859	A	U	C(a,g)
1059:1198	U:A(g)	C:G	C:G
1115:1185	C:G	G:C	C:G
1118:1155	C:G	U:A	U(g):A(c)
1159	A	U	U
1297	C	U	A(u,g)

* Numbering is according *Escherichia coli* nomenclature.

† All 16S rRNA sequences of 'green non-sulfur bacteria and relatives' are from the most recent versions of the Ribosomal Database Project and GenBank.

the genus *Oscillochloris*. However, additional phenotypic studies are necessary.

The *Oscillochloris* strains showed only remote relatedness with non-phototrophic members of the phylum (69.2–76.6 % sequence similarity). The phototrophic genera *Oscillochloris*, *Chloroflexus* and *Heliothrix* formed a monophyletic cluster in which the highest level of sequence similarity was found between branches of *Chloroflexus* and *Oscillochloris* (82.5–86.5 %). However, a similar level of sequence similarity had been shown for members of related families: for example, *Chromatiaceae* and *Ectothiorhodospiraceae* have 84–90 % similarity (Imhoff & Suling, 1996) and

Haloanaerobiaceae and *Halobacteroidaceae* show 82–85 % similarity (Rainey *et al.*, 1995). In our opinion, the level of sequence divergence of *O. trichoides* DG-6^T as well as the new isolates and other phototrophic genera of the phylum corresponds to an interfamilial one.

More detailed analysis of the 16S rDNA sequences produced 'signatures' of base substitutions that delineated the *Oscillochloris* and *Chloroflexus* phylogenetic clusters (Table 2). The secondary-structure analysis shows that most of these changes occurred in helices and involved paired bases (compensatory substitutions). Moreover, the *Oscillochloris* and other

Table 3. Comparative characteristics of *O. trichoides* DG-6^T and *C. aurantiacus* OK-70fl

Characteristic	<i>Oscillochloris trichoides</i>	<i>Chloroflexus aurantiacus</i>
Cell diameter (µm)	0.8–1.0	0.5–1.0
Motility	Gliding	Gliding
Sheath	+	+ / –
Gas vesicles	+	–
Chlorosomes	+	+
Colour of culture	Green	Orange–green
Bacteriochlorophylls	<i>c</i> , <i>a</i>	<i>c</i> , <i>a</i>
IR max. of bacteriochlorophylls	748, 852	750, 805, 860
Major carotenoids	β-Carotene and γ-carotene	β-Carotene and γ-carotene
Optimum pH	8.0–8.5	7.0–7.8
Temperature category	Mesophilic	Thermophilic
Photolithoautotrophy	+	+
Autotrophic CO ₂ -fixation pathway	Calvin cycle	3-Hydroxypropionate cycle
Electron donors	H ₂ , S ^{2–}	H ₂ , S ^{2–}
Oxidation products of sulfide	S ⁰	S ⁰
Carbon compounds photometabolized	Acetate, pyruvate	Wide range of organic substrates
Tricarboxylic acid cycle	–	+
Glyoxylate shunt	–	+
Assimilatory sulfate reduction	–	+
Reserve compounds	Poly-β-hydroxybutyrate, polyphosphate	Poly-β-hydroxybutyrate, polyphosphate
Saturated/unsaturated fatty acid	0.63	3.3–3.8
Major quinone	MK-10	MK-10
N ₂ fixation	+	–
DNA G + C content (mol %)	59.2	57.6
16S rDNA G + C content (mol %)	60.4–61.3	62.8–63.9

phototrophic branches of the dendrogram should be characterized by specific versions of regions corresponding to positions 70–100, 820–880 and 1149–1180 of *Escherichia coli*.

Taking into account the deep branching point of organisms of the genus *Oscillochloris* within the 'green non-sulfur bacteria and their relatives' phylum and the fact that they are well defined phenotypically and phylogenetically, we propose the family *Oscillochloridaceae* fam. nov. with a single genus, *Oscillochloris*, for mesophilic filamentous gliding phototrophic bacteria. Table 3 summarizes the main properties of *O. trichoides* DG-6^T and *C. aurantiacus* OK-70fl.

Therefore, we propose the following taxonomic changes:

1. To create the new family *Oscillochloridaceae* with the type genus *Oscillochloris*.
2. To emend the description of the genus *Oscillochloris*.
3. To emend the description of the species *Oscillochloris trichoides*; according to Rules 18a and 18f of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992), to designate strain DG-6^T

as the type strain of *Oscillochloris trichoides*, instead of the description used before as the type strain of this species.

Description of *Oscillochloridaceae* fam. nov.

Oscillochloridaceae (Os.cil.lo.chlo.ri.da'ceae. L. part. adj. *oscillans* oscillating; Gr. adj. *chloros* green; M.L. fem. pl. n. *Oscillochloridaceae* taxonomic family name of the oscillating green bacteria).

Cells are arranged in multicellular flexible filaments, of constant diameters, with gliding motility. The trichomes do not form branches and are uniformly wide throughout their length. The length of trichomes is variable, with or without sheaths. Trichomes multiply by the separation of short segments or single cells from the parental trichome. Cells may contain gas vesicles. Trichomes appear green or yellowish-green. The cells contain bacteriochlorophylls *c* and *a* as well as carotenoid pigments. The light-harvesting structures of the photosynthetic apparatus are chlorosomes. Photolithoautotrophic or photolithoheterotrophic under anoxic conditions. Some strains are capable of growth under microaerobic conditions. All known species are mesophilic. The differences in the sequences of 16S rRNA between members of the

families *Oscillochloridaceae* and *Chloroflexaceae* are in the range 13.5–17.5%. The type genus is *Oscillochloris* (Gorlenko and Pivovarova 1977, 1989).

Emended description of the genus *Oscillochloris* (Gorlenko and Pivovarova 1977, 1989).

Oscillochloris [Os.cil'lo.chlo'ris. L. part. adj. *oscillans* oscillating; Gr. adj. *chloros* green; M.L. fem. n. *Oscillochloris* oscillating green (bacterium)].

Cells are arranged in uniseriately multicellular flexible filaments with gliding motility. Trichomes are uniformly wide throughout their length, length-variable, non-branching and are with or without sheaths. Trichomes multiply by the separation of short segments or single cells from the parental trichome. Cells usually contain gas vesicles. Cells stain Gram-positive or Gram-negative. Trichomes appear green or yellow-green. The cells contain bacteriochlorophylls *c* and *a* as well as carotenoids. The light-harvesting structures of the photosynthetic apparatus are chlorosomes. All known species are mesophiles. Photolithoheterotrophic or photolithoautotrophic under anaerobic conditions; capable of photosynthesis in the presence of hydrogen sulfide, during which they deposit elemental sulfur globules outside the cells. Some strains are capable of growth under microaerobic conditions. The G + C content of the DNA of *Oscillochloris chrysea* was not determined. For *O. trichoides* strain DG-6^T, the G + C content of the DNA is 57.3–59.2 mol% (melting point). The type species is *Oscillochloris chrysea* (Gorlenko and Pivovarova 1977, 1989).

Emended description of *Oscillochloris trichoides* [Gorlenko and Korotkov 1979, 1989; *Oscillatoria trichoides* (Szafer) Lauterborn 1915]

Oscillochloris trichoides (tri.cho'i.des. Gr. adj. *trichoides* hair-like).

Trichomes are straight or wave-like, green and 0.8–1.4 µm wide. Individual cells in filaments vary in length from 2.0 to 5.0 µm. There may or may not be a thin sheath or trichomes are surrounded by a thin slime layer. Cells are motile by slow gliding at a rate of 0.2 µm s⁻¹ and multiply by separation of a short section of the filament or of an individual cell from the parental trichome. In older cultures, gas vesicles are formed along the cell septa. The light-harvesting structures are chlorosomes. Bacteria deposit poly-β-hydroxybutyrate inside the cells. Strain DG-6^T is strictly anaerobic and obligately phototrophic. Cell suspensions appear dark green when grown under anaerobic conditions in the light. Some strains can grow under microaerobic conditions and appear orange. The *in vivo* absorption spectra of cell suspensions have maxima at 333, 456, (520), 748 and 852 nm. The major photosynthetic pigment is bacteriochlorophyll *c*; a small amount of bacteriochlorophyll *a* is also present. The major carotenoid pigments are β-carotene

and γ-carotene. Strain DG-6^T contains menaquinone (MK-10) as the only quinone. Mesophilic, with optimal growth at 28–30 °C. Cells grow at pH 6.8–9.0, with an optimum at pH 8.0–8.5. Growth under photolithoautotrophic conditions can be detected in the presence of sulfide and/or molecular hydrogen as the electron donors. The optimal concentration of sulfide (Na₂S.9H₂O) is 0.5–0.7 g l⁻¹. Cells are tolerant to sulfide at concentrations up to 1.5 g l⁻¹. Cells oxidize sulfide to elemental sulfur, which is deposited outside the cells. Carbon dioxide fixation proceeds via the Calvin cycle. Cells grow preferentially on medium containing acetate, pyruvate, casein hydrolysate or yeast extract in addition to bicarbonate and sulfide. Formate, propionate, butyrate, malate, succinate, fumarate, citrate and benzoate do not sustain growth. The TCA cycle is truncated, lacking 2-oxoglutarate dehydrogenase. The key enzyme of the glyoxylate bypass (isocitrate lyase) is absent. Sulfide, cysteine, cystine and sulfur, but not sulfate, can be used as sulfur sources. The cells use ammonium salts, urea, glycine, glutamate, glutamine, asparagine and casein hydrolysate as nitrogen sources. The capacity of the strain DG-6^T to fix molecular nitrogen was confirmed by the ability of the cell suspension to reduce acetylene. Yeast extract or a mixture of vitamins (thiamin, biotin, riboflavin and folic acid) is required for growth. The habitat is mats on the surface of sulfide-containing mud in freshwater pools and springs. In the environment, these species coexist with other anoxygenic phototrophic micro-organisms (*Chlorobium* sp., *Allochrochromatium* and *Thiocapsa* species) and cyanobacteria (*Oscillatoria* and *Pseudanabaena* species). The G + C content of the DNA of the type strain is 59.2 mol% (melting point). The type strain is DG-6^T (= VKM B-2210^T; All Russian Collection of Microorganisms, Pushchino, Moscow Region).

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