The global atmospheric level of methane (CH$_4$), the second most important greenhouse gas, is currently increasing by ~10 million tons per year. Microbial oxidation in unsaturated soils is the only known biological process that removes CH$_4$ from the atmosphere, but so far, bacteria that can grow on atmospheric CH$_4$ have eluded all cultivation efforts. In this study, we have isolated a pure culture of a bacterium, strain MG08 that grows on air at atmospheric concentrations of CH$_4$ [1.86 parts per million volume (p.p.m.v.)]. This organism, named *Methylocapsa gorgona*, is globally distributed in soils and closely related to uncultured members of the upland soil cluster $\alpha$. CH$_4$ oxidation experiments and $^{13}$C-single cell isotope analyses demonstrated that it oxidizes atmospheric CH$_4$ aerobically and assimilates carbon from both CH$_4$ and CO$_2$. Its estimated specific affinity for CH$_4$ ($a^\alpha$) is the highest for any cultivated methanotroph. However, growth on ambient air was also confirmed for *Methylocapsa acidiphila* and *Methylcapsa aera* close relatives with a lower specific affinity for CH$_4$, suggesting that the ability to utilize atmospheric CH$_4$ for growth is more widespread than previously believed. The closed genome of *M. gorgona* MG08 encodes a single particulate methane monooxygenase, the serine cycle for assimilation of carbon from CH$_4$ and CO$_2$, and CO$_2$ fixation via the recently postulated reductive glycine pathway. It also fixes dinitrogen and expresses the genes for a high-affinity hydrogenase and carbon monoxide dehydrogenase, suggesting that atmospheric CH$_4$ oxidizers harvest additional energy from oxidation of the atmospheric trace gases carbon monoxide (0.2 p.p.m.v.) and hydrogen (0.5 p.p.m.v.).

methylene | USC alpha | trace gases | isolate | filter cultivation

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**Significance**

Increasing atmospheric methane concentrations contribute significantly to global warming. The only known biological sink for atmospheric methane is oxidation by methane oxidizing bacteria (MOB). Due to the lack of pure cultures, the physiology and metabolic potential of MOB that oxidize atmospheric methane remains a mystery. Here, we report on isolation and characterization of a MOB that can grow on air and utilizes methane at its atmospheric trace concentration as a carbon and energy source. Furthermore, this strain has the potential to utilize five additional atmospheric gases, carbon dioxide, carbon monoxide, hydrogen, nitrogen, and oxygen to supply its metabolism. This metabolic versatility might be the key to life on air and this discovery is essential for studying the biological methane sink.

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This article is a PNAS Direct Submission. This open-access article is distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND). Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession no. CP024846).

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**Widespread soil bacterium that oxidizes atmospheric methane**

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MOB were not shown to grow at atmospheric CH₄ concentrations and likely rely on storage compounds during periods of low CH₄ availability that are built up during periodic exposures to high concentrations of CH₄ (6, 24).

Research on USC clades spans nearly two decades. As no cultured representatives are available and even their 16S rRNA genes remained unknown until very recently (25), these bacteria could be traced in the environment only by using the gene encoding a subunit of the particulate methane monoxygenase (pmoA) as a specific marker (16, 18, 24, 26, 27). As revealed in pmoA-based studies, USCcs occur exclusively in soils, with USCα being found primarily at pH neutral and acidic conditions, while USCγ is thriving mostly in pH neutral and alkaline soils (8, 16). Notably, both groups have also been observed in arctic upland soils (28). During the last years, information on their distribution, abundance, and activity was supplemented with first insights into their genomic repertoire as obtained from metagenomics (25, 29–31), but no complete genome sequence of a USC member has yet been reported.

In this study, we report the isolation of a pure culture of a member of the USCα clade, strain MG08, the basic morphological and physiological traits of this bacterium, and its complete genome sequence. Microcolony-growth experiments on floating filters revealed that our isolate grows on polycarbonate and inorganic filters floating on nitrate mineral salt medium (NMS) under an atmosphere of ambient air. Combined genomic and proteomic analyses revealed that the cultured USCα strain encodes and expresses an enzymatic repertoire for using CH₄, carbon monoxide, and hydrogen, suggesting that the latter two trace gases provide additional energy sources. We explicitly showed oxidation of atmospheric CH₄ by Methylcapsa gorgona MG08 and estimated that it has a much higher specific affinity (a') for CH₄ than other available MOB isolates (23). However, cultivation of other MOB on floating filters under an atmosphere of ambient air showed that strains with a lower specific affinity for CH₄ were also able to grow, questioning whether a "high affinity" or "high-specific affinity" for CH₄ is the decisive prerequisite for an oligotrophic life on air.

Results and Discussion

After 2 y of enrichment a pure culture of a MOB from a landfill soil inoculum was obtained. The complete genome sequence of strain MG08 was determined from 94,080 long reads (Pacific Biosciences) which assembled into a single circular chromosome with 3,326,440 bp and a GC content of 58.94% (SI Appendix, Table S2). All reads mapped to the genome confirming that no contaminating organisms were present in the culture. The genome encoded 46 tRNA genes, a single 16S, 23S, and 5S rRNA gene operon, a single pmoCAB operon, and one orphan pmoC gene.

Phylogeny and Global Distribution.

The inferred pmoA sequence of the newly cultured MOB group within the previously described USCα Jasper Ridge 1/cluster 5 (32) (Fig. 1A) and had up to 87.7% amino acid identity with environmentally retrieved sequences (FR720169) within this cluster. Consistently, 16S rRNA gene-based phylogenetic analyses showed that this MOB clusters with the 16S rRNA gene sequence (97.8% identity) of the uncultured USCα Candidatus Methylloaffinis lahnergensis (25) (SI Appendix, Fig. S1). Two additional USCα genomes that were recently assembled from subarctic mire metagenomes did not contain 16S rRNA genes (31). The average amino acid identity (AAI) between our isolate and previously published USCα metagenome-assembled genomes (MAGs) ranges from 70% to 72% (Fig. 2A) confirming that our isolate and existing USCα MAGs belong to the same genus. Interestingly, the AAI between our isolate and characterized Methylcapsa species ranges from 67% to 69%. This is comparable to the AAI shared within validly published Methylcapsa species (67–72%), suggesting that our isolate and, by extension, other USCα MAGs are all members of the genus Methylcapsa. Genomic average nucleotide identity (gANI) shared between our isolate and other members of the Methylcapsa genus, including the USCα MAGs, ranges from 74% to 79%, demonstrating that the isolate constitutes a species within the genus Methylcapsa (Fig. 2A), which we named M. gorgona MG08. gorg.gona. L. fem. n. gorgona (from Gr. fem. n. gorgone) a vicious female monster from Greek mythology with sharp fangs and hair of living, venomous snakes in reference to the hair-like structures produced by the type of strain (Fig. 1B).

The identification of M. gorgona as a member of the USCα group was confirmed by phylogenetic analysis based on a concatenated alignment of 34 conserved taxonomic marker genes (Fig. 2B). However, we did not recover strong support for a monophyletic clade of Methylcapsa, regardless of whether we considered USCα as part of the genus or not. This lack of resolution was due to the inclusion of two strains of Methyllocella...
in our phylogenetic reconstruction. These strains

Methylophaga capsulata B2 (GCA 000247445.1)
Methylophaga aurea KTG (GCA 0074106.1)
Methylophaga palmarum NE2 (GCA 000114285.1)
Methylophaga silvestris B2 (GCA 00021745.1)
Methylophaga silvestris TFC (GCA 00021745.1)
Methylophaga gorgona MGG07 (This Study)

Fig. 2. Average nucleotide and amino acid identities, phylogenetic relationship, and central metabolism comparison between M. gorgona MG08 and its genome sequenced relatives. (A) Symmetrical matrix of pairwise gANI and AAs between all strains and MAGs and ordered as in B. ANI is presented in the Lower Left triangle and values ≥74 are provided. AAI is presented in the Upper Right triangle and values ≥60 are provided. M. gorgona MG08 and Ca. M. lahnbergenis (AAI, 71.3; ANI, 78.1), MAG USCI (AAI, 70.1; ANI, 78.0), MAG USCA (AAI, 70.0; ANI, 77.3), M. aurea KTG (AAI, 69.3; ANI, 72.5), M. capsulata B2 (AAI, 67.4; ANI, 74.75), M. palmarum NE2 (AAI, 66.1; ANI, 67.9) and M. silvestris B2 (AAI, 62.5; ANI, 69.9) are below the species threshold of 96.6 ANI (3) and 95 AAI (4). (B) The phylogenomic tree was calculated with 10 independent chains of 11,000 generations under the LG model with four rate categories, using an input alignment of 34 concatenated marker genes (Materials and Methods). A total of 6,000 generations of each chain were discarded as burn-in, the remainder were subsampled every five trees (bpcomp -x 6000 5 11000) and pooled together for calculation of the reported 50% consensus tree and bipartition posterior probabilities (maxdiff = 0.814, meandiff = 0.010076). The model and number of rate categories was identified using ModelFinder (Materials and Methods). (C) Distribution of functional complexes presented in Fig. 4 and SI Appendix, Table S1 were determined using blast (5), OrthoFinder (6), and manual examination of trees. Presence of a complete complex is indicated by a solid square. Complexes that are incomplete are indicated with an embedded diamond. Abbreviations for functional complexes: aca, carbonic anhydrase; acc, acetyl-CoA carboxylase; atp, ATP synthase; cox, carbon monoxide dehydrogenase; cyd, terminal cytochrome oxidase; eno, enolase; fad, formaldehyde activating enzyme; fdh, formate dehydrogenase; fdx, ferredoxin, 2Fe–2S; fhe, formyltransferase/hydrolase complex; fhf, formate–tetrahydrofolate ligase; FNR, ferredoxin-NADP+ oxidoreductase; fol, bifunctional 5,10-methylene-tetrahydrofolate dehydrogenase, and 5,10-methylene-tetrahydrofolate cyclohydrolase; gck, 2-glycerate kinase; gcv, glycine cleavage complex; gly, serine hydroxymethyltransferase; hof, hydroxypropionate reductase; mch, methenyl tetrahydromethanopterin cyclohydrolase; mcl, mali-Coa lyase; mdh, malate dehydrogenase; mtd, NAD(P)-dependent methylene tetrahydromethanopterin dehydrogenase; mtka, malate thiokinase; mxa, methanol dehydrogenase; nif, nitrogenase; nuo, NADH:quinone oxidoreductase; pet, ubiquinol-cytochrome c reductase; pmo, particulate methane monooxygenase; ppc, phosphoenolpyruvate carboxylase; and sga, serine–glyoxylate aminotransferase.

silvestris in our phylogenetic reconstruction. These strains share 64–65% AAI with recognized species of Methylocapsa, which is lower than the 67–72% AAI shared within recognized Methylophaga but similar to the 63–69% AAI shared between recognized species of Methylophaga and USCα genomes (Fig. 24). Together, Methylophaga, USCα, and Methylocella all form a single strongly supported clade. Although AAI values and phylogenetic arguments taken together support the assignment of M. gorgona MG08 and other USCα as members of the Methylophaga, our data do not resolve the relationship between Methylocella and lineages of Methylophaga. We hope that the isolation and genome sequencing of additional Methylophaga strains in the future will help resolve the branching order and relationships within this clade.

Screening of all publicly available environmental 16S rRNA gene amplicon datasets identified 194,764 sequences in 1,537 datasets (0.8% of all screened datasets) that were identical or very similar (≥97% identity) to the 16S rRNA gene of M. gorgona MG08. These datasets originated almost exclusively from terrestrial environments (primarily from soil) spanning six continents, with latitudes ranging from the high arctic Svalbard over the tropics to southern Australia and New Zealand (Fig. 3), demonstrating that microbes identical to or highly related to our isolate are globally distributed in terrestrial ecosystems.

Comparative Genomics and Proteomics. The genome of M. gorgona MG08 encodes and expresses several pathways that are typically found in alphaproteobacterial MOB, but excitingly also contains unique metabolic features (Figs. 2C and 4 and SI Appendix, Table S1). Like other MOB it uses a particulate methane monooxygenase (pMMO) for CH4 oxidation to methanol. pMMO requires copper, but the methanobactin (a copper-binding peptide) operon found in various Methylocystis species (33) is not present in the genome of M. gorgona MG08. However, we detected a homolog to one member of this operon, the methanobactin biosynthesis cassette protein MbnB/DFUF692 (PF05114.12). In M. gorgona MG08 and other Methylophaga species, this gene is part of a conserved gene cluster of four genes homologous to the GIG operon in Legionella pneumophila which responds to altered copper concentrations (34). These genes encode a putative integral membrane signal protein (DUF2282–PF10048.8, expression confirmed in proteome in the presence of 1 μM Cu), a putative DNA binding protein (DUF2063–PF09836.8), and an inner membrane protein (DoxX domain containing PF07681). We observed a PQQ-linked methanol dehydrogenase (MxaFJGI)
which uses calcium as a catalytic cofactor to convert methanol to formate. MG08 might encode this pathway for formate oxidation, which is absent in the closely related USCs (Dataset S1 and S2).

MG08 encodes a complete pathway for tetrahydrodromethanopterin (H₂MPT)-mediated formaldehyde oxidation to formate, a nonreversible formate dehydrogenase for NADH generating formate oxidation to CO₂, and the pathway for C₁ incorporation in the serine cycle via tetrahydrofolate (H₄F)-mediated C₁ transfer (Fig. 4). A putative pathway of formaldehyde oxidation to formate via H₂F also exists. This pathway requires spontaneous conversion of formaldehyde to 5,10-methylene H₂F for its operation (37). However, based on previous experiments with M. extorquens, where both pathways are present but only the H₂MPT pathway is responsible for formaldehyde oxidation, we consider it unlikely that the H₂F pathway is responsible for formaldehyde oxidation in M. gorgona MG08. We also identified gene sets for a complete oxidative tricarboxylic acid cycle (TCA), the Entner–Doudoroff pathway, the pentose phosphate pathway, and the glyoxylate cycle, as well as a complete respiratory chain with five versions of terminal respiratory oxidases, indicating that it has the ability to grow under different oxygen concentrations (Fig. 4 and SI Appendix, Table S1).

The genome did not encode a complete ethylomalonyl-CoA pathway. M. gorgona MG08 has an incomplete Calvin–Benson–Bassham (CBB) cycle and therefore contrasts in this respect with other members of *Methylocapsa*, *Methylocella*, and *Methylophaga* which have a complete reductive glycine pathway for CO₂ reduction (45). This suggests that *M. gorgona* MG08 has lost the gene for the canonical Rubisco. This is not a general feature in USCs as the USCS1 and USCS2 MAGs (Dataset S1) and the type IV Rubisco, while the genes for the canonical Rubisco and its type IV homolog were not found in the incomplete genome of Ca. *M. lahnbergensis* (25). Further insights into the distribution of the canonical RuBisCO must await the complete genome sequence of *Ca. M. lahnbergensis* and other USC strains. *M. gorgona* MG08 does have a complete reductive glycine pathway for CO₂ fixation (46) which includes a formate dehydrogenase complex homologous with the oxygen-tolerant complex in *Rhodobacter capsulatus* that was experimentally confirmed to catalyze CO₂ reduction (SI Appendix, Fig. S2B) (47). This pathway overlaps with the H₂F-mediated C₁ transfer for simultaneous assimilation of CO₂ and ammonia (NH₃). Genes for this pathway were consistently found in all genomes of the genera *Methylocapsa*, *Methylocella*, and the three USC MAGs, but of the three MAGs only Ca. *M. lahnbergensis* contained all necessary genes for the complete pathway (Dataset S1). In addition, *M. gorgona* MG08 possesses the complete *nifH* and *nifKX* operon that codes for structural nitrogenase components, which, with the exception of the single gene *nifH* in USC1 is absent from the draft MAG of Ca. *M. lahnbergensis* USC1 and USC2 (Fig. 2C and Dataset S1), NiFe and NiFd phylogenies showed that the *nif* genes of *M. gorgona* MG08 have a different evolutionary origin than those of other closely related cultures (SI Appendix, Fig. S3). Genes encoding hydroxylamine oxidoreductase (*huo*) and cytochrome *c₅₅₉* were absent from the genome, confirming that *M. gorgona* MG08 does not have the potential to derive energy from ammonia oxidation (48). Interestingly, *M. gorgona* MG08 contains genes that encode an O₂-resistant/insensitive [NiFe] group 1 high-affinity respiratory hydrogenase (*hhyL* and *hhyS*) and a [MoCu] class I respiratory carbon monoxide dehydrogenase (Fig. 2C and SI Appendix, Fig. S4). As these enzymes belong to the same lineages as those encoded by nonmethanotrophic communities in Antarctic desert soils (49) and the soil actinobacterium *Mycobacterium smegmatis* (50) that are capable of using atmospheric concentrations of H₂ and CO, *M. gorgona* MG08 might
were manually |

were oxidized and 

were grown in am-

oxidation at both high and low CH

MOB. We therefore performed experiments to test

concentrations using a microcolony cultivation

and MOB

concentration using a microcolony cultivation

MG08 grown in liquid cul-

MG08 phylogenetically 

∼

while smaller colonies formed under

concentrations.

head-

stimulated growth.

Microcolonies of

SYBRgreen and incubated for 10 min,

M. gorgona

confirmed after 5 and 12 mo (Fig. 5), reaching more than 10 times

Continued growth of

whether this isolate can grow on ambient air containing an at-

Air - 26 days

Air - 5 months

Air - 12 months

20 ppm CH4

17 days

1000 ppm CH4

17 days

be able to conserve energy from aerobic respiration of these trace 
gases at atmospheric concentrations as well. This feature is obviously 
more widespread among USCa members as the draft genomes of 
Ca. M. lahnbergensis, USC1 and USC2 also encode closely related CO 
dehydrogenases (Fig. 2C and SI Appendix, Fig. S4B), and USC1 and 
USC2 (the incomplete genome of Ca. M. lahnbergensis lacks a high-
affinity hydrogenase) contain hydrogenase genes that are highly 
similar to the one found in M. gorgona MG08 (Fig. 2C and SI Ap-
pendix, Fig. S4A).

Proteomic analyses of M. gorgona MG08 grown in liquid cul-
ture under an atmosphere containing 20% CH4 (but at ambient 
concentrations of CO and H2) in the presence of 10 mM nitrate 
certain that all proteins shown in Fig. 4 except nitrogenase 
and a cbb3 type cytochrome c oxidase were expressed (SI Ap-
pendix, Table S1). Among the detected proteins were those of 
the reductive glycine pathway, including its CO2 reductase, the 
high-affinity hydrogenase, and carbon monoxide dehydrogenase.

Physiology and Morphology. M. gorgona MG08 phylogenetically 
belongs to the clade of putative high-affinity alphaproteobacterial 
USCa MOB. We therefore performed experiments to test whether 
this isolate can grow on ambient air containing an at-
mospheric CH4 concentration using a microcolony cultivation 
technique of filters floating on liquid nitrate mineral salt medium 
without any added energy or carbon source. These experiments 
equivocally demonstrated that M. gorgona MG08 grew in ambient 
air (~1.86 p.p.m.v. CH4, ~0.2 p.p.m.v. CO, and ~0.5 p.p.m.v. 
H2) and higher (20 and 1,000 p.p.m.v.) CH4 concentrations (Fig. 5).

After up to 3 wk of incubation the largest colonies were observed 
under 1,000 p.p.m.v. CH4 while smaller colonies formed under 
20 p.p.m.v., similar to those in unamended atmospheric air, 
confirming that increased concentrations of CH4 stimulated growth. 
Continued growth of M. gorgona MG08 in unamended air was 
confirmed after 5 and 12 mo (Fig. 5), reaching more than 10 times

its initial population size after 5 mo and continuing to grow and 
form spherical colonies observable after 12 mo (Fig. 5). In compar-
ison, starving Sinorhizobium melliloti were able to triple their 
inital population size using intracellular polyhydroxybutyrate 
(PHB) as a carbon and energy source and maintain its population 
for 5 mo before it declined to a level below its initial size (51). A 
species more closely related to M. gorgona MG08, the MOB 
Methylocystis parvus OBDP, did not replicate at all using stored 
PHB and depended on the access to CH4 for co-oxidation of the 
two substrates for growth (52). Considering this, we find it im-
ausible that M. gorgona MG08 would depend entirely upon PHB 
for its growth over the course of 12 mo, but it is possible that any 
existing PHB storages were tapped during the incubation period 
and used as a carbon and energy supplement to its main diet.

M. gorgona MG08 encodes only a single copy of the particulate 
methane monooxygenase which was also detected in its proteome. 
This shows that the same enzyme is responsible for 
catalyzing CH4 oxidation at both high and low CH4 concentrations. 
To confirm that M. gorgona MG08 is able to oxidize atmospheric 
concentrations of CH4 we performed a CH4 oxidation and 
microcolony-growth experiment with cells on floating filters 
under atmospheric air (1.86 p.p.m.v. CH4). The results clearly 
show that CH4 was oxidized during the 120 d of incubation under 
ambient air (Fig. 6A). Inspection of the filters confirmed cell 
growth (Fig. 6B). M. gorgona MG08 also carries single copies of 
the high-affinity respiratory hydrogenase and carbon monoxide 
hydrogenase and both were expressed under 20% CH4 head-
space concentrations, without added CO or H2. Thus, it seems 
likely that these proteins are constitutively expressed at different 
CH4 concentrations and thus contribute to the energy harvest 
from air. However, it is not possible to conclude on this matter 
until we have successfully determined the proteome of M. gorgona 
MG08 grown on air, which was not yet achieved due to biomass 
limitations. Furthermore, due to the difficulty in preparing 
headspace atmospheres and media free of trace amounts of

![Fig. 5. Microcolonies of M. gorgona MG08 cultivated at different CH4 concentrations. Microcolonies were grown on polycarbonate filters floating on liquid nitrate mineral salt medium for the number of days specified on each picture, either in closed jars with air amended with different concentrations of CH4, or exposed to unamended air. For fixation, the filters were transferred to fresh-made 2% paraformaldehyde in 1x PBS in the refrigerator overnight. For staining, filters were transferred (side with bacteria up) on top of 200 µL droplets of 1,000X SYBRgreen and incubated for 10 min, washed, and air dried.](image-url)

![Fig. 6. CH4 oxidation by M. gorgona MG08 microcolonies incubated on floating filters under atmospheric air (A). Microcolony formation under atmospheric air (B). (A) Five 170-mL bottles with floating polycarbonate filters were incubated on 35 mL 1/10 diluted nitrate mineral salt media (Materials and Methods) under atmospheric air (135 mL 1 atm headspace, sealed with rubber stopper) for 120 d. An additional set of five bottles was incubated without filters as a negative control for CH4 oxidation. A two-sample t test assuming equal variances confirmed that headspace CH4 concentrations were significantly different in bottles with filters containing cells (P value < 0.001), compared with those without cells. (B) Filters from A were manually inspected to identify colony formation. (Top) Showing single cells before incubation. (Bottom) Colonies formed after 120-d incubation in one of the bottles from A. For staining in B, filters were transferred (side with bacteria up) on top of 200 µL droplets of 1,000X SYBRgreen and incubated for 10 min, washed, and air dried.](image-url)
and energy sources such as CO (0.2 p.p.m.v. in air) and H₂ (0.5 p.p.m.v. in air), and EDTA (53) (1.1 μM in the 1/10 diluted nitrate mineral salt medium used), respectively, we cannot yet conclude whether growth is possible solely on atmospheric CH₄. However, growth did occur on high-purity carbon-free alumina matrix Anodisc inorganic filters floating on nitrate mineral salt medium under atmospheric air (SI Appendix, Fig. S5). This confirms that the cells at least did not depend on bisphenol A (BPA) leaking from the polycarbonate filters (54, 55) as an additional carbon and energy source.

An additional CH₄ oxidation experiment, using cells in liquid culture under a range of CH₄ concentrations, was performed to estimate the half saturation constant [Kₘ(app)] for M. gorgona MG08 (SI Appendix, Fig. S6). We show that it has a Kₘ(app) of 4.905 μM, similar to that observed for Methylocapsa acidiphila B2, M. parvus, Methylocystis sp. SC2, and many other MOB strains (23). In contrast to M. gorgona MG08, neither of these strains was previously found to grow at atmospheric CH₄ concentrations. The specific affinity [aₗ] = Vₘ(ax)(app)/Kₘ(app) was suggested as a better measure for oligotrophy (6) as Methylocystis sp. LRI displayed high-affinity activity (Kₘ of ~100 nM) at low CH₄ concentrations (<275 p.p.m.v.) and low-affinity activity (Kₘ of ~1 μM) when CH₄ concentrations were higher while its specific affinity remained constant (56). With a Vₘ(ax)(app) of 9.54 × 10⁻¹² mol·L⁻¹·h⁻¹, the specific affinity of M. gorgona MG08 is ~195 (x 10⁻¹² L·cell⁻¹·h⁻¹), which is ~17 times higher than the corresponding value of Methylocapsa acidiphila B2 (12 × 10⁻¹² L·cell⁻¹·h⁻¹), ~10 times higher than for Methylocystis sp. LRI (20 × 10⁻¹² L·cell⁻¹·h⁻¹), and ~6 times higher than for Methylocystis sp. SC2 (34 × 10⁻¹² L·cell⁻¹·h⁻¹), formerly the MOB with the highest known aₗ (23). The high-affinity MOB is thus probably better defined as a high-specific affinity MOB as suggested by Dunfield (6) more than 10 y ago, but a confirmation of this must await standardized comparisons between USCα and other MOB strains.

Despite having lower specific affinities (aₗ) than M. gorgona MG08, we decided to test whether other Methylocapsa species are able to grow at atmospheric CH₄ concentrations. Surprisingly, both M. acidiphila and Methylocapsa aurica were able to grow on filters incubated under atmospheric air (SI Appendix, Fig. S7). This suggests that the ability of MOB to grow on filters floating on nitrate mineral salt medium under anaerobic air is not a unique capability of species within the USCα, and not dependent on high aₗ for CH₄. However, the apparent success of USCα in many upland soils may still be a result of having the highest aₗ.

Consistent with the physiological and genomic predictions, NanoSIMS experiments performed on microcolonies on polycarbonate filters qualitatively showed that M. gorgona MG08 incorporated¹³C-labeled carbon into its biomass when grown in a closed jar containing 20 p.p.m.v. ¹³CH₄ and unlabeled CO₂ (Fig. 7A). Incorporation of ¹³C was also observed in the presence of ¹²CO₂ at 20 p.p.m.v. and unlabeled CH₄ (Fig. 7B), as expected from CO₂/¹²CO₂ incorporation via the carboxylation reaction of the serine cycle (enzyme no. 16, Fig. 4). There is also the possibility that CO₂ could be assimilated via the reductive glycine pathway, but with the current data the different possible entry points of carbon assimilated from CO₂ cannot be distinguished. Accumulation of ¹³CO₂ in the headspace of the cultures during incubation with 20 p.p.m.v. and 1,000 p.p.m.v. ¹³CH₄ confirmed that the organism is able to oxidize CH₄ completely to CO₂ and subsequently release CO₂ from the cell (SI Appendix, Fig. S8).

Additional physiological characterizations of M. gorgona MG08 were carried out in liquid cultures grown at a high CH₄ concentration. Efficient growth was observed when N₂ was offered as the sole nitrogen source under fully aerobic conditions (SI Appendix, Fig. S9). M. gorgona MG08 shares this ability with the closely related M. acidiphila B2¹, and nonmethanotrophic members of the genus Beijerinckia, while Methylocystis and Methylosinus species require a somewhat reduced oxygen tension and Methylococcus capulatus (Bath) is highly sensitive to oxygen (39, 57, 58). Almost all tested nitrogen containing compounds including N₂, NO₂⁻, and NH₄⁺ acted as good nitrogen sources for M. gorgona MG08 (SI Appendix, Fig. S9), while histidine and glycine inhibited growth as shown previously for M. capsulatus (only histidine) (59), Thiobacillus neapolitanus (only histidine) (60), and methylotrophic bacteria (only glycine) (61).

No growth was observed in liquid batch culture controls provided with acetate, ethanol, formate, galactose, glucose, methanol, oxalate, pyruvate, sucrose, succinate, or urea without added CH₄ (SI Appendix, Fig. S10A). To evaluate potential concentration effects of methanol, we attempted growth on 5%, 0.5%, 0.01%, 0.005%, and 0.001% CH₄, but observed only minimal increases in the optical density of the cultures (SI Appendix, Fig. S10B). Considering that M. gorgona MG08 carries and expresses the genes for methanol oxidation, its inability to grow on methanol was surprising but it is in line with the observations that M. aurica, M. acidiphila, and Methylocapsa palsaum grow poorly on methanol (39, 62, 63). Interestingly, we observed a concentration-dependent growth inhibition of M. gorgona MG08 in the presence of methanol concentrations ≥0.01% when growing on CH₄ (20% CH₄ in headspace). Thus, toxicity alone could not explain its inability to use methanol. Perhaps methanol dehydrogenase, which requires oxidized cytochrome c for accepting electrons, thus depends on simultaneous CH₄ oxidation (Fig. 4) due to tightly coupled CH₄ and methanol oxidation reactions occurring within a supercomplex. If so, the redox interactions would be different from intracytoplasmic membrane assemblies of pMMO and methanol dehydrogenase (MDH) in the methanol and CH₄ utilizing M. capsulatus (Bath) (64).

The incapability of M. gorgona MG08 to grow on acetate was also surprising, considering that it carries all of the genes required for aerobic metabolism of acetate, i.e., acetate-CoA ligase, acetate kinase, phosphotransacetylase, and a complete TCA cycle. Similarly, M. acidiphila B2¹ also possesses the full set of enzymes for acetate metabolism (65), but is unable to grow on acetate as a sole carbon source (39). Dedyh et al. (66) reported similar findings for the obligate methanotroph Methylomonas stellata AR4⁴ and suggested that the lack of ability to utilize acetate might be due to an absence of a specific acetate/glycolate transporter gene, acpT, rather than the quantity of membrane transporter genes (67, 68). In support of this hypothesis, the acpT gene encoding acetate permease is absent both in strain M. gorgona MG08 and M. acidiphila B2¹.
The cells of *M. gorgona* MG08 are Gram-negative, nonmotile cocccoids, or thick rods that occur singly or in conglomerates. Cells are 0.6-0.8 μm wide and 0.8-1.5 μm long and show numerous hair-like structures (Fig. 1B). Growth on surfaces occurs by formation of microcolonies of a circular form. It reproduces by normal cell division and does not form rosettes. Cells contain a well-developed intracytoplasmic membrane system of type III inclusions packed in parallel on only one side of the cell membrane (Fig. 1C). This arrangement has been shown to be characteristic for members of the *Methylocapsa* genus (39, 62, 63). Inclusions resembling PHB granules were present (Fig. 1C) but did not exhibit the bipolar arrangement of refractile PHB granules characteristic of *M. aurea* KAY7' and members of the *Methyl-ocella* genus. Optimal growth was observed between 15 °C and 27 °C, while some growth still occurred at the extremes tested, 7 °C and 37 °C (SI Appendix, Fig. S11A). The optimum growth temperature range was similar to but wider than *M. aurea* (25–30 °C), and higher/wider than that of *M. acidiphila* (20 °C) and *M. p slamming* (18–25 °C) (39, 62, 63). *M. gorgona* MG08 is thus far the only *Methylocapsa* strain capable of growth at 37 °C. The optimum pH for growth of *M. gorgona* MG08 was in the range of 6.5 to 7 (SI Appendix, Fig. S11B), considerably higher than for *M. acidiphila* (5.0–5.5), *M. aurea* (6.0–6.2), and *M. p slamming* (5.2–6.5), but in line with the frequent detection of USC members of the *Methylococcus* genus. Optimal growth was observed between 15 °C and 27 °C (SI Appendix, Fig. S11C), similarly to the three other *Methylocapsa* strains (39, 62, 63).

**Conclusions**

We have obtained a pure culture of an organism growing on ambient air containing an atmospheric methane concentration and named it *M. gorgona* MG08. *M. gorgona* MG08 utilizes CH4 as a carbon and energy source and we have experimentally demonstrated that it can utilize at least three additional constituents of air: CO2 as an additional carbon source, N2 as a nitrogen source, and O2 as terminal electron acceptor. Furthermore, it carries and expresses the genes necessary to exploit two additional atmospheric components, CO and H2, as energy sources. Such metabolic flexibility may account for the enigmatic lifestyle of atmospheric CH4 oxidizers and explain how they can grow on air alone. However, *M. gorgona* MG08 also grows efficiently at high CH4 concentrations, but expresses only one pMMO, demonstrating that this enzyme is responsible for CH4 oxidation at high and low concentrations. In line with this, *M. gorgona* MG08 has the highest specific affinity (αCH4) for CH4 of any known methane oxidizing bacterium, resulting from a high Vmax(app) despite a high Km(app). Furthermore, relatives within the genus *Methylocapsa* previously thought not to live on trace gases were shown to be able to grow on air as well, indicating that this ability is more widespread than previously believed. The isolation and characterization of a member of these recalcitrant organisms have direct implications for our understanding of the biological sink of atmospheric CH4 and how atmospheric trace gases support life on our and possibly other planets.

**Materials and Methods**

**Enrichment, Isolation, and Cultivation.** The sampling site for *M. gorgona* MG08 was cover soil in a ditch in a retired subarctic landfill in northern Norway (69°20′ N 18°59′ E). Five grams of soil was added to 50 mL nitrate mineral salt medium (0.1x NMS) at pH 6.8 (DSHM medium 921). Samples were shaken and streaked onto Whatman polycarbonate filters (Nucleopore filters) for preparation of filters. The filters were then transferred to Petri dishes with 20 mL liquid NMS medium and floated with the bacteria on the upper side. The dishes were either wrapped with parafilm and incubated in ambient air or stacked in 8.5-L jars (BD BBL GasPak 150) and incubated in air to which 20% (vol/vol) CH4 was added. The whole procedure was performed at room temperature and in the dark. A fresh-made anti-fading solution consisting of 0.1% p-phenylenediamine dihydrochloride in 1:1 glycerol and PBS (pH 7.2) was used for mounting the filters on slides with coverslips.

A cell density of 20-50 cells per 50 × 50 μm filter area was selected for microcolony cultivation. A filtration manifold with 10 × 20 mL stainless steel chambers (THI LAB Products) was used for preparation of filters. Each filter was washed with 70% ethanol and rinsed with sterile dH2O before a GF/C filter (25 mm Whatman) was added as support filter. A polycarbonate filter (25 mm Nucleopore 110600) was then added before the chimney was placed on top. Even distributions of cells on the filters were obtained by pouring 12 mL NMS medium into the chimney before mixing in the cells and applying vacuum. The chimney walls were rinsed with 2 × 5 mL sterile water during suction. The polycarbonate filters were then transferred to Petri dishes with 20 mL liquid NMS medium and floated with the bacteria on the upper side. The dishes were either wrapped with paraffin and incubated in ambient air or stacked in 8.5-L jars (BD BBL GasPak 150) and incubated in air to which CH4 was added to obtain 20, 50, or 1,000 p.p.m.v. CH4 in the headspace. The filters were harvested after 17 d and transferred to freshly made 2% paraformaldehyde in 1× PBS and stored in the refrigerator. Prior to microscopy, filters were washed with 0.1% sodium dodecyl sulfate and 0.02% sodium hypochlorite and 70% ethanol.

Microcolony cultivation was performed following the procedure described above for the *Anodisc* filters. Cultivation experiments were conducted on 2-wk- and 4-wk-old batch cultures grown in sealed vials with 20% CH4 (vol/vol). pH range was tested in NMS medium containing 0.1 M phosphate buffer ranging from pH 5 to pH 9 obtained by mixing 0.1 M solutions of KH2PO4 and K2HPO4 for 7 d. NAD-dependent growth was determined over the range of 0.1–5.0% (wt/vol) for 24 d. Temperature tolerance was tested from 7 °C to 37 °C for 24 d. Methanol utilization was tested over a range of 0.001–5% (vol/vol). The following carbon sources 

(tryptone, glucose, yeast extract, agar (TYG/AJ) with culture aliquots. This was done routinely in addition to microcosm. For cultivation in liquid, 10 mL culture was incubated in 125-mL serum bottles with air containing 20% CH4, as headspace (vol/vol), without shaking. Serum bottles were closed with butyl rubber septa and crimp caps. For cultivation on plates, NMS agar plates were made with bacteriological agar type E (Biokar Diagnostics). Unless otherwise stated, all liquid and plate incubations were done at room temperature (21 °C) in the dark.

We observed a weak, but visible, growth of the culture on NMS agar plates after a 5-min incubation on air. Based on this observation, a series of experiments was initiated. Initially, *M. gorgona* MG08 was inoculated in NMS medium without CH4 addition in Erlenmeyer beakers, with paraffin lids, to which sterile distilled water was added every 2–4 wk to compensate for evaporation. Other incubations were done in serum bottles with 20 p.p.m.v. to 20% CH4 in the headspace (vol/vol). In the cultures with less than 1,000 p.p.m.v. CH4 in the headspace, there was no increase in optical density during the course of 8 mo of incubation. *M. gorgona* MG08 cells were nevertheless still alive after these long incubations, evidenced by rapid increase in optical density following their transfer to liquid medium with 20% CH4 (vol/vol) in the headspace. As growth of *M. gorgona* MG08 on air could not be demonstrated by standard cultivation in liquid media, microcolony-growth experiments on polycarbonate filters floating on liquid NMS medium were performed.

A culture of *M. gorgona* MG08 was incubated for 2 mo in liquid NMS with 200 p.p.m.v. CH4 in the headspace as start culture for microcolony growth on filters. The cell concentration in the suspension was determined by fluorescence microscopy after filtration on Anodisc filters (Whatman 6809-6022) and 1,000× SYBRgreen I (Molecular Probes S-7563) staining. For staining, filters were washed (transferred bacteria side up) on top of 200-μL droplets of 1,000× SYBRgreen and incubated for 10 min. In the next step, filters were washed twice by transferring them onto 1 mL milliliter water and then air dried. The fluorescence was determined using a microplate reader.

Microscopy was performed using an Axio Observer Z1 inverted microscope (Carl Zeiss Microscope) equipped with a HBO 100 W mercury lamp for excitation and a SYBERgreen filter. Cells were visualized by spinning disk confocal microscopy using an Axio Observer Z1 inverted microscope (Carl Zeiss Microscope) equipped with a CSU-X1 scan head (Yokogawa) and a 100× NA1.4 plan-apochromat objective lens. SYBRgreen fluorescence was excited by the 488-nm laser line and collected through a 500- to 550-nm bandpass filter. Z stacks were captured with a Rolera EMCCD camera (QImaging) using ZEN software (2.3 (Carl Zeiss Microscope), deconvolved in Huygens ver. 14.10 (SVI) using a theoretical point spread function, and finally exported as maximum intensity projections. An Axio Observer Z1 microscope with filter cube 38 was used for routine observations. Scanning electron microscopy (SEM) was performed at the Electron Microscope Laboratory, UiT The Arctic University of Norway. SEM samples were fixed and prepared as described previously (63) and scanned with a Carl Zeiss Sigma Field Emission scanning electron microscope.
were tested at a concentration of 0.05% (wt/vol): acetic, ethanol, formate, galactose, glucose, methanol, oxalate, pyruvate, succinate, sucrose, and urea. Utilizable non-alkaline sources were assessed by replacing KNO₃ in liquid NMS medium with 0.05% (wt/vol) l-alanine, l-arginine, l-asparagine, l-aspartate, l-cysteine, l-glutamate, l-histidine, l-isoleucine, NaHCl, yeast extract, or peptone. Growth on nitrogen sources was compared with growth in nitrogen-free NMS medium to assess N₂-fixation capability. Nitrogen fixation was further tested in nitrogen-free medium under microoxic (5% O₂ (vol/vol) with liquid comprising half volume) vs. fully oxic conditions (150 rpm). Experiments were conducted on cells grown in liquid NMS medium with 20% (vol/vol) methane in the headspace. Growth was monitored for 30 d, unless otherwise stated, by optical density comparison with a respective control at 600 nm using a Spectramax 250 microplate spectrophotometer system (Molecular Devices).

**CH₄ Oxidation in Atmospheric and Artificial Artificial and Inorganic Filter Controls.** Five 170-ml bottles with floating polycarbonate filters were incubated on 35 mL 1/10 diluted NMS (diluted with phosphate buffer pH 6.8) under atmospheric air (135 mL 1 atm headspace, sealed with rubber stopper) for 120 d. An additional set of five bottles was incubated without filters as a negative control for CH₄ oxidation. All filters had ~150-250 cells per 50 × 50 μm. For CH₄ measurements, 0.5 mL of headspace gas was sampled with a gastight gas chromatograph (GC) syringe (Hamilton). The contained gas was injected manually into a GC Fisher Scientific 1310 with column TG-BOND M512A. Detection was achieved with a flame ionization detector (FID) with a detection limit of ~0.5 ppm CH₄. Standards at 1.8 p.p.m. (air) and 10 p.p.m. CH₄ were checked at startup every day, and regularly during each batch of measurements. All calculations of actual concentrations accounted for the removal of gas for measurements. Growth tests were also performed on ultrapure carbon-free Anodic inorganic filter membranes (25 mm, pore size 0.2 μm; Whatman) and polycarbonate filters (25 mm; Nuclepore 110606). All experiments were conducted using cells from the same batch of culture (NMS), preincubated for 2 mo under a headspace atmosphere containing atmospheric air and 200 p.p.m. CH₄. Filters with cells were prepared as described above for microcolony cultivation. Diluted NMS was used to avoid previously experienced issues with crystal formation on filters due to high salt concentrations. No reductions in growth resulting from dilution were observed.

**CH₄ Oxidation Experiment in Liquid Culture for Estimation of Kinetics Parameters.** From log-phase batch cultures cultivated at 21 °C under 20% headspace CH₄ concentrations in liquid NMS medium, all cultures for the CH₄ oxidation experiments were prepared. Triplicate cultures with 21.6 mL of 5 × 10⁶ cells per milliliter (culture diluted to the right density with phosphate buffer, pH 6.8) and one negative control with sterile NMS diluted with phosphate buffer (pH 6.8) were prepared for each of the following CH₄ concentrations: 823 p.p.m. headspace (1.26 μM dissolved), 0.23% (3.5 μM), 0.77% (11.8 μM), 2.2% (33 μM), 3.9% (59 μM), 5.1% (77.2 μM), and 6% (92.2 μM). All 28 samples were incubated for 8 h at 21 °C. CH₄ concentration was measured, and optical density was measured at time (T) 0, 2.5 h, 5 h, and 8 h. For CH₄ measurements, headspace gas was sampled with a calibrated 1-mL gas syringe (VICI) with a side-port needle (0.020 × 0.012 × 2 inches; VICI). The pressure in the syringe was adjusted to ambient pressure, and the contained gas was injected into a gas chromatograph (SRI 8610C, FID configuration). At each time point, 340 μL of the culture was sampled in parallel. The optical density was determined by measurement using a Spectra Max 250 microplate reader, Molecular Devices at 410 nm (NMS diluted with phosphate buffer as blank). Cell densities were converted to cell numbers by separate standards for each culture, where the spectrophotometric density measurements of cell culture dilution series were associated to cell counts obtained using counting chambers. To account for the proportion of dissolved gases at 21 °C, masses of CH₄ were calculated from Henry’s law, assuming an ideal state, knowing the ambient pressure, temperature, and headspace volume of the bottle at ambient pressure, liquid volume, mixing ratio of CH₄ and the temperature-dependent solubility constant. All calculations accounted for removal of gas and liquid for measurements. V_morph (μm³), and associated SEs of their means were estimated by nonlinear regression using the R-package nlstools and its built-in functions.

**Morphological Observations.** All morphological tests were conducted on 2-wk- and 4-wk-old batch cultures grown in liquid NMS in sealed vials with 20% CH₄ (vol/vol). Cell morphology and results of the Gram-stain reaction were determined by light microscopy. Lysis in 0.2% and 2% (vol/vol) SDS and lysis in 3% KOH was measured using an inoculum loop to assess colony viscosity (70). The presence of PHB inclusions was identified by visual inspection of transmission electron micrographs and confirmed by staining with Nile blue A (71). Exospore formation was observed after passage using a 100 μm BG-12 filter. Cultivation was done on Gelrite and Wys (73) and by phase-contrast microscopy for stationary-phase liquid and plate cultures.

**NANO-SIMS.** Microcolony cultivation with either 13C4CH₄ or 13CO₂ (in the presence of the corresponding unlabeled gas) was done at 20 p.p.m. CH₄ headspace concentration. Before use, the polycarbonate filters were coated with a gold-palladium (AuPd) alloy thin film (nominal thickness approximately 30 nm) in a low vacuum sputter coater utilizing argon as the working gas. The start culture consisted of a cell culture of M. gorgona MG08 grown in liquid NMS with a headspace atmosphere consisting of 200 p.p.m. CH₄ in ambient air. The cell density for NanoSIMS was five times higher than the density for microcolony cultivation. The cells were filtered onto the AuPd-coated filters in the way as described above. The filters were transferred to Petri dishes containing 20 mL liquid NMS medium pH 6.8 with the filters floating bacterial side up. Cultivation was done in 8.5 L jars to which gas volume was added by syringe. The filters were harvested at day 17 and fixed in the fridge overnight on freshly prepared 2% paraformaldehyde in 1× PBS. Using the vacuum filtration unit, filters were washed with 10 mL 1/2× PBS and then 10 mL miliQ. The filters were then left floating on miliQ water for 2× for 1 min before drying in darkness on glass slides shielded with plastic frames. For NanoSIMS analysis, 7 × 7 mm square sections were cut from the central area of the filters and attached to antimony-doped silicon wafer plates (Active Business Company) with commercially available superglue (Loctite, Henkel). The samples were then coated with an additional AuPd film (nominal thickness 30 nm) because some of the films deposited on the filters before the incubation provided insufficient electrical conductivity for charge compensation in the NanoSIMS measurement process.

**NanoSIMS measurements were performed on a NanoSIMS NS50L instrument (Cameca).** Data were acquired as multilayer image stacks by scanning a finely focused Cs+ primary ion beam over areas between 45 × 45 and 50 × 50 μm² with a 512 × 512 pixel image resolution and approximately 80 nm physical resolution (probe size). The applied primary ion beam current and dwell time were 2.0 pA and 10 ms/layer (pixel), respectively. The detectors of the multilayer assembly were positioned for parallel detection of 13C/12C, 13C/12C, 13C/12C, 13C/12C, 13C/12C, 13C/12C, 13C/12C, 13C/12C, and 13C/12C secondary ions. The mass spectrometer was tuned to achieve mass resolving power (MRP) of >9,000 for detection of 12C+ and >10,000 for 13C secondary ions (MRP according to Cameca’s definition). Carbon isotopomer distribution about 13C/12C was measured for each additional images. Signal intensities were inferred from simultaneously recorded secondary electron intensity distribution images.

**Analyses were performed either on entire cells (long-term acquisition runs) or**

**by sampling partial cell volumes, in both cases accessed by presputtering with a slightly defocused, high-intensity primary ion beam.** Data comparison was performed on image data acquired from regions close to the center of the cells which were reached after irradiation of paraformaldehyde-fixed M. gorgona MG08 cells with 16 keV C⁶⁺ primary ions at a fluence (dose density) of 3 × 10¹⁰ ions/mm².

**Image data were evaluated using the WinImage software package provided by Cameca (version 2.0.8).** Before stack accumulation, the individual images were aligned to compensate for positional variations arising from primary ion beam and/or sample stage drift. Secondary ion signal intensities were dead time corrected on a per-pixel basis. Carbon isotope composition images were generated by calculation of per-pixel 13C/12C isotope fraction values, given in atom percent (at%), from the C⁶⁺ and C⁷⁺ secondary ion signal intensities via 13C/12C (13C + 12C) and 13C/12C (2 × 12C + 13C), respectively. All carbon isotope composition data given in the paper were inferred from C⁶⁺ signal intensities owing to the superior counting statistics of this secondary ion species. We did not observe any significant differences between isotope fraction values inferred from C⁷⁺ or C⁶⁺ signal intensities. The estimations and calculations ratio of C⁷⁺ (or C₈⁺) to C⁶⁺ (or C₇⁺) were calculated to continuous-flow gas isotope ratio mass spectrometry (Delta V Advantage, Thermo Fisher Scientific).
Hydrogenases were classified using the web tool HydBDB (87). Heme-copper cytochrome c oxidases were classified using the web tool HCO (88). Homologs to genes encoding the central metabolism of M. gorgona MG08 were identified in related species and MAGs using BLAST (73), OrthoFinder (89), and manual curation of results.

Protein Extraction and Mass Spectrometry. Proteomics was conducted on a 2-wk-old batch culture grown in sealed vials with 20% CH₄ (vol/vol). Proteins were extracted from pelleted cells using SDS buffer (0.1 M Tris-HCl, pH 7, 1.25% w/vol SDS, 20 mM dithiothreitol) and ultrasonication for 3 min (80% power, 0.8 amplitude) as described (90). Protein pellets were dissolved in 20 μL SDS sample buffer (2% SDS, 2 mM beta-mercaptoethanol, 4% glycerol, 40 mM Tris-HCl pH 6.8, 0.1% bromophenol blue), heated to 90 °C for 5 min and separated on a 1D SDS gel. After a short run (10 min) the gel was stained with Coomassie G-250 staining (Merck). The stained gel band was cut, destained, and incubated with trypsin (Promega) overnight at 37 °C. The resulting peptides were desalted using C₁₈ ZipTip column (Merck Millipore) and subsequently dissolved in 0.1% formic acid before liquid chromatography mass spectrometry analysis (nanoLC-MS/MS). Peptide lysates (5 μL) were first loaded for 5 min on the precolumn (μ-precolumn, Acclaim PepMap, 75-μm inner diameter, 2 cm, C₁₈, Thermo Fisher Scientific), at 4% mobile phase B (80% acetonitrile in nanopure water with 0.08% formic acid) and 96% mobile phase A (nanopure water with 0.1% formic acid), and then eluted from the analytical column (PepMap Acclaim C₁₈ LC Column, 25 cm, 3 μm particle size; Thermo Fisher Scientific) over a 150-min linear gradient of mobile phase B (4–55% B). Mass spectrometry was performed on a Q Exactive HF mass spectrometer (Thermo Fisher Scientific) with a TriVersa NanoMate (Advion, Ltd.) source in LC-chip coupling mode as described (91).

Proteome Discoverer (v1.4.0.288, Thermo Fisher Scientific) was used for protein identification and the MS/MS spectra acquired were searched with Sequest HT against the complete genome sequence for strain M. gorgona MG08. Enzyme specificity was selected as trypsin with up to two missed cleavages allowed, using 10-ppm peptide ion tolerance and 0.05-Da MS/MS tolerances. Oxidation at methionine as the variable modifications and carbamidomethylation at cysteines as the static modification were selected. Only peptides with a false discovery rate (FDR) <1% calculated by Percolator (92) were considered as identified.

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1. Prather MJ, Holmes CD (2017) Overexplaining or underexplaining methane’s role in climate change. Proc Natl Acad Sci USA 114:5324–5326.
2. IPCC (2013) Climate Change 2013: The Physical Science Basis, Contribution of Working Group I to the Fifth Assessment Report of the IPCC (Cambridge Univ Press, Cambridge, UK).
3. Dlugokencky EJ, Nisbet EG, Fisher R, Lowry D (2011) Global atmospheric methane: Budget, changes and dangers. Philos Trans A Math Phys Eng Sci 369:2058–2072.
4. Kirchse 5, et al. (2013) Three decades of global methane sources and sinks. Nat Geosci 6:813–823.
5. Conrad R (2009) The global methane cycle: Recent advances in understanding the microbial processes involved. Environ Microbiol Rep 1:285–292.
6. Dunfield PF (2007) The soil methane sink. Greenhouse Gas Sinks, eds Reay DS, Watson FG, Coakley JS, ed. (CABI, Wallingford, UK), pp 152–170.
7. Shallcross DE, Butenhoff CL (2007) The atmospheric methane sink. Greenhouse Gas Sinks, eds Reay DS, Watson FG, Coakley JS, ed. (CABI, Wallingford, UK), pp 171–184.
8. Kienf C (2015) Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on pm0α as molecular marker. Front Microbiol 6:1346.
9. Hanson RS, Hanson TE (1996) Methanotrophic bacteria. Microbiol Rev 60:439–471.
10. Trotsenko YA, Murrell JC (2008) Metabolic aspects of aerobic obligate methano- trophy. Adv Microb Ecol 63:183–229.
11. Sohngen NL (1906) Ueber Bakterien welche Methan als Kohlenstoffnahrung und Energiequelle gebrauchen. Zentral Bakteriol Abt I 5:153–157.
12. Bender M, Conrad R (1992) Kinetics of CH₄ oxidation in soils exposed to ambient air or high CH₄ mixing ratios. Environ Microbiol 2:1061–1069.
24. Degelmann DM, Borken W, Drake HL, Kolb S (2010) Different atmospheric methane-oxidizing communities in European beech and Norway spruce soils. Appl Environ Microbiol 76:3228–3235.

25. Pratscher J, Vollmers J, Wiegand S, Dumont MG, Kaster A-K (2018) Unravelling the identity, metabolic potential, and global biogeography of the atmospheric methane-oxidizing Candidatus Methylocystis. Environ Microbiol 20:1016-1029.

26. Kolb S, Knief C, Dunfield PF, Conrad R (2005) Abundance and activity of uncultured methanotrophic bacteria involved in the consumption of atmospheric methane in two forest soils. Environ Microbiol 7:1150–1161.

27. Brahms PK, Kirtman AE, Legat K, Davis D, Liesack W (2012) Linking composition, activity and seasonal dynamics of atmospheric methane oxidizers in a meadow soil. ISME J 6:1115–1126.

28. Martineau C, et al. (2014) Atmospheric methane oxidizers are present and active in mountain high Arctic soils. FEMS Microbiol Ecol 89:257–269.

29. Edwards CR, et al. (2017) Draft genome sequence of uncultured upland soil cluster Gamma proteobacteria gives molecular insights into high-affinity methanotrophy. Genome Announc 5:e00474-17.

30. Singleton CM, et al. (2018) Methanotrophy across a natural permafrost thaw environment. ISME J 12:2454–2458.

31. Horz HP, Rich V, Ahravan S, Bojanian BH (2005) Methane-oxidizing bacteria in a California upland grassland soil: Diversity and response to simulated global change. Appl Environ Microbiol 71:6463–6462.

32. Semrau JD, et al. (2013) Methanobactin and MmoO work in concert to act as the ‘copper-switch’ in methanotrophs. Environ Microbiol 15:3077–3086.

33. Mironovski K, et al. (2017) The Legionella pneumophila GltG operon responds to gold and copper in planktrophic and biofilm cultures. PLoS One 12:e0171425.

34. Mary CJ, et al. (2012) Complete genome sequences of six strains of the genus Methylocystus. J Bacteriol 194:4768–4774.

35. Vu HN, et al. (2016) Lanthanide-dependent regulation of methanol oxidation systems in Methylobacterium extorquens AM1 and their contribution to methanol growth. J Bacteriol 198:1225–1239.

36. Kallen RG, Jenkins WP (1966) The mechanism of the condensation of formaldehyde and hydrogen to formate. J Biol Chem 241:5851–5863.

37. Chen Y, et al. (2010) Complete genome sequence of the aerobic facultative methanotroph Methylophilus albus. Environ Microbiol 12:2511–2521.

38. Miroshnikov KK, et al. (2017) Draft genome sequence of Methylocapsa palmarum NE2-1, an obligate methanotroph from subarctic soil. Genome Announc 5:e00054-17.

39. Krasagis K, Ooo DM, Jetten MSM, Sinninghe Damste JS, Ettwig KF (2014) Autotrophic carbon dioxide fixation in the Calvin-Benson-Bassham cycle by the denitrifying methanotroph “Candidatus Methyliornisibacter oxyfera”. Appl Environ Microbiol 80:2451–2457.

40. Vorobev AV, et al. (2011) Methylocorallia stellata gen. nov., sp. nov., an acidophilic, obligately methanotrophic bacterium that possesses only a soluble methane monoxygenase. J Bacteriol 193:1255–1260.

41. Chen Y, et al. (2010) Complete genome sequence of the aerobic facultative methanotroph Methylobacterium silvestris B2L. J Bacteriol 192:3840–3841.

42. Kradem AF, et al. (2011) Autotrophic methanotrophy in verrucomicrobia. Methylocystus parasiticus sp. nov. (class Coriobacteria; order Verrucomicrobiales) uses the Calvin-Benson-Bassham cycle for carbon dioxide fixation. J Bacteriol 193:4438–4446.

43. Tabita FR, Hanson TE, Satagopan S, Witte BH, Kreul NE (2008) Phylogenetic and evolutionary relationships of Rubisco and the Rubisco-like proteins and the functional lessons from new modes of carbon dioxide fixation. J Bacteriol 190:2569–2576.

44. Figueroa IA, et al. (2018) Metagenomics-guided analysis of microbial chemo-lithoautotrophic phosphate oxidation yields evidence of a seventh natural CO2 fixation pathway. Proc Natl Acad Sci USA 115:692–6101.

45. Hartmann T, Lehn H, Kolb S (2013) The oxygen-tolerant and NAD+–dependent formate dehydrogenase from Rhodobacter capsulatus is able to catalyze the reduction of CO2 to formate. FEBS J 280:6083–6096.

46. Klotz MG, Steins LY (2008) Nitrogenases of Dicytoceles chilensis involved in cyanobacterial communities in permafrost. J Bacteriol 190:2640–2643.

47. Ji M, et al. (2017) Atmospheric trace gas transport support primary production in Arctic desert surface soil. Nature 552:400–403.

48. Greening C, Beppu J, Harms K, Cook GM, Conrad R (2014) A soil actinobacterium scavenges atmospheric nitrogen using two membrane-associated, oxygen-dependent [NiFe]-hydrogenases. Proc Natl Acad Sci USA 111:4257–4262.

49. Ratcliff WC, Kadam SV, Denison RF (2008) Poly-3-hydroxybutyrate (PHB) supports survival and reproduction in starving rhizobia. FEMS Microbiol Ecol 65:391–399.

50. Peja AS, Sundström CR, Criddle CS (2011) Poly-3-hydroxybutyrate metabolism in the type II methanotroph Methylocystus parvus. Appl Environ Microbiol 77:6012–6019.

51. Hohlfeld R, Lauffer M, Goodhue CT (1975) Degradation of ethylenediaminetetraacetic acid by microbial populations from an aerated lagoon. Appl Environ Microbiol 29:787-794.

52. Hohlfeld R, Lehn H, Kolb S (2013) The oxygen-tolerant and NAD+–dependent formate dehydrogenase from Rhodobacter capsulatus is able to catalyze the reduction of CO2 to formate. FEBS J 280:6083–6096.

53. Klotz MG, Steins LY (2008) Nitrogenases of Dicytoceles chilensis involved in cyanobacterial communities in permafrost. J Bacteriol 190:2640–2643.

54. Greening C, Beppu J, Harms K, Cook GM, Conrad R (2014) A soil actinobacterium scavenges atmospheric nitrogen using two membrane-associated, oxygen-dependent [NiFe]-hydrogenases. Proc Natl Acad Sci USA 111:4257–4262.

55. Ratcliff WC, Kadam SV, Denison RF (2008) Poly-3-hydroxybutyrate (PHB) supports survival and reproduction in starving rhizobia. FEMS Microbiol Ecol 65:391–399.

56. Peja AS, Sundström CR, Criddle CS (2011) Poly-3-hydroxybutyrate metabolism in the type II methanotroph Methylocystus parvus. Appl Environ Microbiol 77:6012–6019.

57. Hohlfeld R, Lauffer M, Goodhue CT (1975) Degradation of ethylenediaminetetraacetic acid by microbial populations from an aerated lagoon. Appl Environ Microbiol 29:787-794.

58. Hohlfeld R, Lehn H, Kolb S (2013) The oxygen-tolerant and NAD+–dependent formate dehydrogenase from Rhodobacter capsulatus is able to catalyze the reduction of CO2 to formate. FEBS J 280:6083–6096.