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From nature to nurture: Essence and methods to isolate robust methanotrophic bacteria

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ABSTRACT

Methanotrophic bacteria are entities with innate biocatalytic potential to biofilter and oxidize methane into simpler compounds concomitantly conserving energy, which can contribute to copious industrial applications. The future and efficacy of such industrial applications relies upon acquiring and/or securing robust methanotrophs with taxonomic and phenotypic diversity. Despite several dramatic advances, isolation of robust methanotrophs is still a long-way challenging task with several lacunae to be filled in sequentially. Methanotrophs with high tolerance to methane can be isolated and cultivated by mimicking natural environs, and adopting strategies like adaptive metabolic evolution. This review summarizes existent and innovative methods for methanotrophic isolation and purification, and their respective applications. A comprehensive description of new insights shedding light upon how to isolate and concomitantly augment robust methanotrophic metabolism in an orchestrated fashion follows.

1. Introduction

Since ages, greenhouse gases like carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and chlorofluorocarbons are perpetually being formed and fragmented apart through an interplay of chemical and biological processes. A balanced presence of these greenhouse gases is conducive to the earth's environment to mitigate global warming and other climatic extremities [1,2]. CH_4 emissions have been reckoned from natural wetlands, rice paddies, animal husbandry, biomass burning, landfills, permafrost carbons of arctic lakes and fossils [3,4]. Human induced emissions in pretext of industrialized urbanization led to an augmented accumulation of atmospheric methane [5,6]. CH_4 is a notorious greenhouse gas which leads to a substantial increase in radiative forcing with global warming potential of 80 over 20 years relative to CO_2 [7–9]. Methanotrophs can oxidize and considerably mitigate environmental CH_4 emissions and aerobic methanotrophic bacteria contributes to up to 10–20% of global CH_4 obliteration [10–12].

Isolation, screening and cultivation of robust and/or unexplored methanotrophs from natural habitats is an attractive perspective to resolve global warming in the long-term [13,14]. Methanotrophs are generally resistant to laboratory cultivation, with typically diverse habitat preferences, including boiling acidic springs, alkaline lakes, permafrost thaw, volcanic geothermal/hydrothermal vents, seeps, farmlands, forest marshes, rice paddies, landfills, animal husbandry wastes, burned biomass, and effluent sludge from factories [4,15–17]. General cultivation techniques mimic natural habitat conditions *in vitro*, and include methanotroph culture in growth media within gas-tight vials or seal-tanked plates with provision of CH_4 supply (20–50%) [18,19]. Apart from CH_4 and air, incubation with supplementary gases like CO_2, oxygen (O_2), nitrogen (N_2) and hydrogen (H_2) could stimulate primary and intermediary metabolisms in methanotrophs like *Methylocaldum*, *Methylococcus*, *Methylocella*, *Methyloferula*, *Methylocapsa*, *Candidatus Methylomirabilis oxyfera*, *Methylacidiphilum sp.* and Verrucomicrobial species [20–22]. The compositions of various methanotrophic growth media like nitrate mineral salts (NMS), ammonium mineral salts (AMS), and other improvised/assorted media for inducing specific growth were briefly outlined in the compendium (Table 1). The compendium describes specific cultivation methods particularly designed for target methanotrophs of variable environs/habitats, which dictate acidophilic, neutrophilic, thermophilic or hydrothermophilic cultivation conditions [23–26].

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Table 1
Compositions of various growth media and the relevant target methanotrophs.

Medium	Major Components (g/L)	Target methanotrophs	Ref
Nitrate mineral salts (NMS) medium	KNO_3 1g; $MgSO_4$ $7H_2O$ 1g; $Na_2HPO_4.12\ H_2O$ 0.717g; KH_2PO_4 0.272g; $CaCl_2.6H_2O$ 0.2g, ferric ammonium EDTA 0.004g; distilled water 1000 ml, pH 6.8	Neutrophilic methanotrophs from freshwater and marine environments	[24,97]
Dilute nitrate mineral salts (DNMS) medium	NMS medium diluted 1:5 using distilled water containing 1 mM of NaH_2PO_4–Na_2HPO_4 buffer, pH 5.5–7.0.	Mild acidophiles and neutrophiles from freshwater and salt-free terrestrial environments	[98]
Ammonium mineral salts (AMS) medium	NH_4Cl 0.5g; $MgSO_4$ $7H_2O$ 1g; $Na_2HPO_4.12\ H_2O$ 0.717g; KH_2PO_4 0.272g; $CaCl_2.6H_2O$ 0.2g; ferric ammonium EDTA 0.005g; 1000 ml distilled water, pH 6.8	Neutrophilic methanotrophs from freshwater environments	[97]
Fortified ANMS media	NH_4Cl 0.5g; $MgSO_4$ $7H_2O$1g; $Na_2HPO_4.12\ H_2O$ 0.717g; KH_2PO_4 0.272g; $CaCl_2.6H_2O$ 0.2g; ferric ammonium EDTA 0.005g; 1000 ml distilled water, 30 ml NaCl, 1 mM KH_2PO_4, 500 µM NH_4Cl, 500 µM KNO_3, 1 µM $CuSO_4.5H_2O$, 40 µM FeNaEDTA, 100 nM lanthanides ($LaCl_3$, $CeCl_3.7H_2O$/$NdCl_3.6H_2O$/$PrCl_3$), pH 7.8.	Marine methanotrophs found in lanthanide rich environments	[25]
Low-salt mineral medium (LMM)	KNO_3 0.1g; $MgSO_4$ 0.1g; $CaCl_2.2H_2O$ 0.02g; KBr 0.01g, 100 ml distilled water, 100 µL iron stock solution (Fe–Na-EDTA 4.5 g/L), optional supplements [NH_4Cl (LMM-AC) or $(NH_4)_2\ SO_4$]	Thermophilic methanotrophs of alkaline hot springs	[99]
Assorted medium	0.2 mM $MgCl_2.6\ H_2O$; 0.2 mM $CaCl_2.2\ H_2O$; 1 mM Na_2SO_4; 2 mM K_2SO_4; 2 mM $(NH_4)_2SO_4$, 1 mM $NaH_2PO_4.H_2O$, pH 3.0	Acidophilic methanotrophs of volcanic environments	[100]
Medium 3.9C10.2	NH_4Cl 0.2g; KH_2PO_4 0.05g; $MgSO_4.7H_2O$ 0.02g; $CaCl_2.6H_2O$ 0.01g; Fe-EDTA powder 0.005g; 1000 ml distilled water, pH 3.9	Acidophiles from geothermal soils	[59]
Medium M2	KNO_3 0.25g; KH_2PO_4 0.1g; $MgSO_4.7H_2O$ 0.05g; $CaCl_2.2H_2O$ 0.01g; NaCl 0.02g; 1000 ml distilled water, pH 5.5	Methanotrophs from freshwater wetlands and mildly acidic soils	[101]
Medium N	KH_2PO_4 0.002g; KNO_3 0.001g; NH_4Cl 0.008g; NaCl 0.003g; $CaCl_2.2H_2O$ 0.018g; $MgSO_4$ $7H_2O$ 0.010g; Na_2SiO_3 0.002g; $AlCl_3$ 0.001g; 1000 ml distilled water, pH 5-7	Mild acidophiles from ombrotrophic wetlands	[102]
Medium S	NH_4NO_3 0.5g; $Na_2HPO_4.2H_2O$ 0.5g; KH_2PO_4 0.1g; $FeSO_4.7H_2O$ 0.005g; NaCl 66.0g; Na_2SO_4 10.45g; Na_2CO_3 0.10g; $NaHCO_3$ 0.06g; NaBr 0.28g; $MgCl_2$ 20.89g; $CaCl_2$ 2.21g; 1000 ml distilled water; pH 7.5	Neutrophilic halophiles	[103]
MJmet medium	30 g NaCl, 0.14 g K_2HPO_4, 0.8 g $CaCl_2$, 3.4 g $MgSO_4{\cdot}7H_2O$, 4.18 g $MgCl_2{\cdot}6H_2O$, 0.33 g KCl, 0.25 g NH_4Cl, 0.25 g $NaNO_3$, 0.5 mg $NiCl_2.6H_2O$, 0.5 mg $Na_2SeO_3{\cdot}5H_2O$, 0.1 mg Na_2WO_4, 20 mg $Fe(NH_4)_2(SO_4)_2.6H_2O$, 1000 ml distilled water, 1 ml vitamin solution, 6 mM $NaHCO_3$,1 µM $CuSO_4$, pH 3-7	Hydrothermophilic methanotrophs	[104]

Identification of optimal growth conditions, cultivation media and/or choosing resilient strains can induce superior methanotrophy and curb undesirable bottlenecks [27]. The notion of robust methanotrophy refers to enhanced valorisation of methane (methane-oxidation capacity), which can refer to 'innate robustness' through natural selection or 'induced robustness' through biological alternations made in genetic, metabolic and synthetic pathways of methanotrophs [28,29]. Moreover, it is quintessential to develop efficient tools and strategies to culture and catalogue diverse novel methanotrophs, for harnessing and/or stimulating their metabolic dynamics of CH_4 consumption. Traditional and novel techniques for isolation, cultivation and purification techniques, metabolic evolution methodologies, and their perspectives are comprehensively described in this review. Moreover, adaptive evolution techniques coupled with atmospheric and room-temperature plasma mutagenesis (ARTP) and microbial microdroplet culture system (MMC) are also reviewed as advanced systems to isolate robust methanotrophic strains by inducing tolerance to inhibitors or desired products within limited time frames.

2. Traditional isolation techniques for methanotrophs

Bacterial isolation steps commonly include specimen sample collection from chosen environment, its microscopic examination and diluting/enriching samples before streaking or pour-plating upon agar plates for spatial segregation [30,31]. Enrichment of collected samples in fluids is done to activate the constricted growth of requisite bacteria [32]. Physical isolation of bacteria from their natural abodes and laboratory cultivation can be done providing optimal growth conditions, and methanotrophs require the most finicky cultivation requisites [33]. Since many decades extensive pioneering researches based on methanotroph isolation, characterization, classification and taxonomic frameworks have been carried out by various scientists [34,35]. Isolation of methanotrophs can be done by collecting selective environmental samples and enriching them in supporting growth media [18,36].

Enrichment technique, which is superior to stereotypic direct agar plating (for isolation) involves sequential transfers into fresh medium periodically inducing methanotrophy and inhibiting methylotrophy [37]. A spirillar microaerophilic *Candidatus Methylospira mobilis* sourced from peat soil was isolated through long periods of enrichment. Such spirillar methanotrophs normally elude isolation owing to their peculiar micro-oxic growth requirements. Hence prominence in isolating such kind is that, their helical cellular shape enables rapid motility in waterlogged heterogeneous environments leading to fast cell positioning at desirably high methane and low oxygen settings [38]. *Methylocapsa gorgona* was sourced from landfill soil and isolated through enrichment. It was found to grow aerobically by utilizing methane (as carbon and energy source) in meagre concentrations of atmospheric traces. Moreover, it could also co-metabolize CH_4, CO_2, CO, H_2, N_2, and O_2; thence isolation/discovery of such methanotrophs with metabolic versatility is quintessential to study and comprehend biological methane sinks of the natural ecosystems [39]. A compendium briefing enrichment isolation techniques of various methanotrophic strains from diverse sampling sources, using suitable media and their respective purity testing methods is presented (Table 2).

Large-scale isolation of methanotrophs through enrichment technique can be done in well-equipped bioreactors [40]. A previously uncultured mixture of 'aerobic methanotrophs and associated denitrifying methanotrophs' was isolated through 53-week enrichment of marine water-sample in nitrate rich medium sparged (10 ml/min) with CH_4:CO_2 (95:5 v/v) at 25 °C, 250 rpm in dark using 5.2 L bioreactor [41]. In another study, sourced from volcanic mud, anaerobic methanotrophic archaea (*Methylophaga sp.*, *Methylobacter sp.*) and sulphate reducing bacteria (*Deltaproteobacteria*) were 286-days enriched and isolated in a SR-AOM bioreactor supplied with sulphate and pressurized methane (8 MPa) [42]. Enrichment of freshwater lake sediment samples using a membrane biofilm bioreactor operated for 13 months (10–25 °C) led to isolation of *Candidatus Methylomirabilis oxyfera* featuring methanotrophy and coupled denitrification [43].

Apart from aiding methanotrophic growth, enrichment can also be done to induce, co-production of ectoines [44], co-metabolism of tricholoroethene along with methane [45], and improving temperature tolerance (from 25 °C to 30/37 °C) for accumulation of

Table 2
Comparisons of enrichment isolation techniques of various methanotrophic strains, their sources and purity testing methods.

Isolation technique	Strains	Sources	Purity testing	Ref
Pre-enrichment using NMS medium	*Methylotetracoccus oryzae* strain C50C1	Paddy soil	Phase-contrast and electron microscopy	[1]
Enrichment culturing using MG mineral medium	*Methylovulum miyakonense*, *Methylomonas paludis*	Acidic peat bog sample: Russia	Transmission electron microscopy	[38]
Enrichment culturing using low-salt methanotrophic medium	*Methylococcaceae* strains: BRS-K6, GFS-K6 and AK-K6	Waterlogged rice field soil, methane seep pond sediments: Bangladesh. Warm spring sediments: Armenia	Phase-contrast, transmission electron microscopy	[99]
Enrichment technique using NMS medium with artificial sea water instead of distilled water and sodium nitrate	*Methylocaldum* strain $S8^T$	Marine sediment from hydrothermal vent, Japan	Plating upon NMS agar plates, phase contrast microscopy	[105]
Enrichment technique using NMS medium	*Methylocapsa gorgona* MG08	Subarctic Landfill soil, Norway	plating upon TGYA rich medium (tryptone, glucose, yeast extract, agar), Scanning electron microscopy, confocal microscopy	[39]
Enrichment technique using MJmet medium	*Methylomarinum vadi*	Marine mud sample, Japan	Streaking repeatedly for single colony formation upon gellan gum plates at least six times, electron microscopy	[104]
Enrichment culturing using liquid mineral medium M2	*Methylocystis bryophila* S285	Acidic peat soil, Russia	Iteratively surface plating upon the agar medium M2	[106]
Enrichment culturing using NMS medium	*Methylomonas*, *Methylosinus species*	Peatland plant fragments	Iteratively surface plating upon the NMS agar medium	[58]
Enrichment culturing using NMS medium supplemented with 300 nM $CeCl_3$	*Methylacidiphilum*, *Methylacidimicrobium* strains	Geothermal regions, Russia	Scanning electron microscopy, transmission electron microscopy	[51]

polyhydroxyalkanoates [46]. Lanthanide dependant *Methylosinus sp.* Ce-a6, sourced from a Japanese pond sediment was isolated by enrichment of methane supplied mineral-medium cultures supplemented with cerium chloride. Its dependency on lanthanide chloride enabled growth, utilization of insoluble lanthanide oxides and enhanced survival in circumneutral conditions [47].

'Dilution-to-extinction' culturing is a method of diluting mixed microbial cultures down to extent of merely retaining single cells yielding pure isolates [48], and has been applied for quick methanotroph isolation from pre-enriched cultures [49]. Miniaturized extinction culture performed in 96 microwell plates allowed direct isolation of 11 methanotroph monocultures (*Methylomonas*, *Methylosinus sps.*) from 4 enriched environmental samples [50]. Thermoacidophilic verrucomicrobia (*Methylacidiphilum*, *Methylacidimicrobium* sps.) were also isolated through enrichment followed by dilution-to-extinction-culturing [51]. Several-month enrichments followed by carbon isotopic incubation experiments led to isolation of 'aerobic methanotrophs (*Methylococcales*) and anaerobic methanotrophic archaea' sourced from methane hydrates of seafloor sediments. Here dilution-to-extinction experiments were adopted for sampling small volumes of surface sediments [52].

Samples collected from environs rich in methane were subjected to enrichment in continuous stir tank bioreactors supplied with uninterrupted methane-air concoctions, using gradually (step-wise) increasing dilution rates up to threshold wash-out stage. This resulted in eventual shifts in overall microbial community growth, and preferential isolation and recovery of the methanotrophic population community. Gammaproteobacterial methanotrophs of *Methylomonas* and *Methylosarcina* genera could be recovered predominantly through this bioreactor operation employing increased dilution rates as confirmed by 16SrRNA amplicon sequencing [53]. Enrichment technique is a time-consuming process with variations in time limits depending upon physiologies of the targeted methanotroph isolate, and can be used with bulk samples; however, though dilution-to-extinction is rapid and reliable, it is suitable for straightforward isolation of quickly thriving methanotrophs, applicable even for samples of small volumes [18,50]. Hence a combination of these two natural methanotroph isolation techniques, along with regular serial dilution and agar-plating, could help achieve apposite results curbing underlying demerits.

3. Modern isolation techniques for methanotrophs

Development and adoption of modern isolation and cultivation techniques like ARTP, MMC, high throughput technologies, single cell isotope imaging, mass-spectroscopy and adaptive metabolic evolution could further help to comprehend and exploit bioprocesses and metabolites of the methanotrophic cultivation, thereby increasing the incidence of novel methanotroph isolation [53–56]. The purity and taxonomic identity of a methanotroph can simultaneously be determined by elaborate molecular identification techniques like 16S rRNA sequencing, T-RFLP analysis, pmoA/mmoX PCR cloning, $^{13}CH_4$ stable-isotope probing and FISH analysis [57–60]. Some uncultured methanotrophs of unrecognized geothermal ecosystems cannot be identified as methane-oxidisers by comparing with 16S rRNA sequences of known methanotrophs. In such cases, enrichment microcosms of methane-oxidizing geothermal samples can be evaluated in the terms of methane oxidizing capacity and activity at extreme temperatures (37–75 °C) and transcriptional activity (detection of mRNA-transcripts). This a novel technique of metatranscriptomics for detecting stress-responsive genes expressed in microcosms for adaptation to environmental changes, and helps us comprehend and decipher metabolic pathways in thermophilic methanotrophy [61]. Metagenomic approaches can also be used to guide novel methanotrophic cultivation methods by unearthing methanotrophic phylogenies and metabolic diversities, which can be quantified by relevant genes/pathways [62].

ARTP is a tool featuring whole cell mutations induced by radiofrequency and 'room-temperature and atmospheric plasma (helium-

based) discharges' to enhance medium/nutrient tolerance promoting cellular growth/metabolism [63–66]. ARTP can be combined with supplementary mutational methods like γ-ray and CRISPR/Cas9 for delimiting industrial drawbacks in methanotrophs like *Methylococcus capsulatus* [67,68]. ARTP technique using pressured glow discharges induced genomic mutational breeding in *Methylosinus trichosporium* OB3b [69]. Measurements of intracellular copper uptake inducing mutations in *Methylococcus capsulatus* were done through inductively coupled plasma mass spectroscopy (ICP-MS). It was also deciphered that when grown in copper-limited conditions the organism secretes 'MopE' protein which can bind with both oxidised and reduced copper, based upon 'X-ray near edge absorption spectroscopy' and 'Electron Paramagnetic Resonance' analyses [70,71].

MMC is an automated platform integrating adaptive evolution with high–throughput microbial cultivation [72,73]. Here microorganisms can be grown in droplets with gaseous exchange facility allowing continuous sub-cultivation and improving robust strain construction under induced stress conditions [72]. Likewise, MMC can be applied for methanotroph growth and cultivation studies also as fluorinated oil-phase micro-droplets can support CH_4 exchange along with O_2 [74]. Methanotrophic strain MeSV2.2 and *Methylobacterium extroquens* AM1 were found to exhibit good growth in MMC. Adaptive evolution experiments of MeSV2.2 by cultivation for 18 days in MMC resulted in a mutant with improved growth rate and high final cell density was desired [72,75].

Uncultivable methanotrophic gamma-proteobacteria like *Crenothrix sp*., which have been evading isolations previously are now being isolated and catalogued taxonomically applying cumulative technologies like Raman-Microspectroscopy, and Microfluidics [54]. Single-cell isotopic imaging is a novel technique featuring stable isotope probing, and application of single-cell-level imaging tools using Raman spectra of individual cells to yield bands for modelling cellular profiles and metabolites with higher throughputs and lower costs [76]. Metagenomic analysis using coupled techniques of stable-isotope-labelling and single-cell-imaging mass spectrometry could elucidate isolation and identification of *Crenothrix* population (major methane metabolisers of stratified lakes), which could grow potentially with and without oxygen supply due to possession of genes encoding typical gamma-proteobacterial 'PmoA' [77]. Mass spectroscopy or gas chromatography can be used for the analysis of fatty acids of whole-cell methanotrophs like *Methylogaea oryzae*, *Methylococcus capsulatus* Bath, *Methyloparacoccus murrellii*, *Methylocaldum gracile* [78]. In cold adapted methanotroph *Methylovulum psychrotolerans*, analysis of fatty acid methyl esters was done using gas chromatography-mass spectrometry, and intact polar-lipid analysis of biomass was done by ultra-high pressure liquid chromatography-high resolution mass spectrometry [79].

High throughput technologies like Omics for molecular analysis of DNA/RNA, proteins and metabolites of methanotrophs can help of comprehend geochemical and communal functions/links of methanotrophy [80,81]. High-throughput cultivation technique applies the dilution-to-extinction-culturing to partition cells singly into tubes/microwells with low nutrient media [50,82]. Due to the immense taxonomical and physiological diversity of methanotrophs isolation discoveries expanding their nomenclatural groups, their identification or cataloguing can be done by the application of high-throughput metagenomic screening/sequencing [83–85]. High-throughput sequencing of 16sRNA gene amplicon regions has been used for identifying differences between methanotrophic family clusters of *Methylococcaceae* and *Methylocystaceae* isolated from diverse ecosystems by multiplex sequencing [86]. Similarly methanotrophic biodiversity of two soil habitats was distinguished and reported using high-throughput sequencing of their pmoA genes [87]. Global methanotrophic diversity of thermoacidiphilic verrucomicrobial species of common ancestral origins are caused by allopatric evolution caused by geographical habitat distances [51].

Adaptive laboratory evolution techniques are methods which promote the growth of methanotrophs by inducing gradual adaptive changes at laboratory level thereby metabolically engineering microbial cells to suit industrial conditions [88,89]. Metabolic adaption is not just phenotypic but genetically stable in methanotrophs as tested in *Methylomicrobium album* BG8 [88]. They are simpler than stereotypic genetic engineering experiments, and use metabolic responses like nutrient limitation and/or induced stress factors to incorporate evolutionary changes [90]. As putrescine is inhibitive for methanotrophy, adaptive evolution was conducted to induce tolerance of *Methylomicrobium alcaliphilum* 20Z to putrescine dihydrochloride (400 mM) [91]. In lactate-tolerant *Methylomonas sp.* DH-1, efficient D-lactate production from CH_4 was induced by adaptive laboratory evolution [92]. Evidently, adaptive laboratory evolution has also been reported for isolating mutants of *Methylobacterium extorquens* AM1 exhibiting stress tolerance to 1-butanol with augmented survival/growth rates, and was confirmed by metabolomics analysis and whole genome sequencing [93]. Adaptive laboratory evolution can also be carried out to induce higher methane degradation rates [56], by incubating the methanotrophic strains of interest at gradually increasing methane contents.

4. Perspectives

Isolation of novel methanotrophs with biotechnological potentials and devising strategies which could address the same, is the need of essence due to their unique metabolisms to devour harmful greenhouse gases like methane. Increasing methane content supply gradually was proven to be a novel technique to amplify innate methanotrophic metabolism and concomitantly free them from unwanted associated heterotrophic methanotrophs/methylotrophs that feed upon methane derivatives like acetate, formate, methanol, succinate or organic acids [94]. Co-occupant satellite species that associate with methanotrophs form functional communities together. Experimental community dynamics reveal that manipulated synthetic environments could be designed more realistically to represent natural processes in laboratory, by studying their proteins, metabolites, transcripts and mutative manipulations [95]. It was within the context of this review to demonstrate that simple isolation methods could be coupled with adaptive evolution techniques to isolate robust high-methane tolerant pure methanotrophic isolates within limited time periods [56]. To summarize, bringing methanotrophs from their natural habitats and nurturing them in our laboratory conditions requires perseverant skill and knowledge of novel reproducible techniques which can be adopted at economic feasibility. Harnessing methanotroph biotechnology for the greater good and welfare of the human society could include pollution control contributing to balanced biogeochemical cycles through bioremediation of xenobiotically contaminated soil/aquatic environments. Application of diverse methanotrophic biofilters for bioconversion of methane-rich abundances like biogas, natural gas, landfill gas and gaseous exhausts from animal husbandry gas, into simpler marketable and transportable commodities, contributes to mitigating green-house gas effects [40,96]. This review paper serves an introductory guide to a researcher wanting to isolate and purify robust methanotrophs in a mediocre laboratory environment with minimal scientific equipment.

CRediT authorship contribution statement

Haritha Meruvu: Conceptualization, Resources, Writing - original draft. **Hui Wu:** Investigation. **Ziyue Jiao:** Validation, Resources. **Liyan Wang:** Resources. **Qiang Fei:** Conceptualization, Supervision, Writing - review & editing.

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