

# Unique and Novel Methanotrophs from Indian Rice Fields and Wetlands: A Documentation of Taxonomic Diversity and a Gateway to Biotechnological, Methane Mitigation and Valorisation Applications

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Review

# Unique and Novel Methanotrophs from Indian Rice Fields and Wetlands: A Documentation of Taxonomic Diversity and a Gateway to Biotechnological, Methane Mitigation and Valorisation Applications

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## Abstract

Methanotrophs represent a distinctive group of microorganisms characterized by their specialized metabolic pathways, unique morphological traits, and diverse taxonomic classifications. These bacteria play a crucial role in the global methane cycle by oxidizing methane, a potent greenhouse gas, thereby contributing to climate regulation. Over the past decade, our research group has made significant strides in exploring methanotroph diversity, particularly within the Indian subcontinent. We have successfully identified and described several novel species and two new genera of methanotrophs isolated from rice fields and wetland ecosystems in India. These discoveries mark the first and only documented methanotrophs from India, highlighting the untapped microbial diversity of these habitats and underscoring the importance of regional studies in global microbial taxonomy. To facilitate these findings, we developed a modified cultivation technique that has proven instrumental in isolating and characterising these elusive organisms. This innovative approach has not only expanded the known diversity of methanotrophs but also opened new avenues for their application in biotechnology. The unique physiological traits of these Indian methanotrophs hold promise for biotechnological innovations, including methane mitigation strategies, biofertilizer development, and bioconversion processes. As climate change continues to pose significant challenges, the valorization of methanotrophs through sustainable technologies presents a compelling opportunity. Our ongoing research aims to harness these microorganisms for environmental and industrial applications, positioning methanotrophs as key players in the pursuit of climate resilience and ecological sustainability.

**Keywords:** methanotrophs; novel; Methylocucumis; Methylobolus; Methylobacter; Methylobacterium

## Introduction

*Methanotrophs: Nature's Methane Managers*

Methanotrophs represent a fascinating and ecologically vital group of microorganisms that play a pivotal role in the global carbon cycle [3]. These bacteria are uniquely equipped to metabolize methane—a potent greenhouse gas—using it as their sole source of carbon and energy [23]. This metabolic specialization not only distinguishes methanotrophs from other microbial groups but also positions them as key players in mitigating climate change [5]. Their distinctive morphology and taxonomy further underscore their uniqueness, with diverse forms adapted to a wide range of ecological niches. In recent years, the study of methanotrophs has gained momentum, driven by the urgent need to understand and harness biological systems that can counteract anthropogenic

environmental impacts [3]. Methane emissions from natural and agricultural sources, particularly rice paddies and wetlands, contribute significantly to global warming [1]. Methanotrophs, by consuming methane before it escapes into the atmosphere, offer a natural solution to this challenge. However, the full potential of these microorganisms remains underexplored due to limitations in cultivation techniques and gaps in our understanding of their diversity and ecological roles [2,19]. Over the past decade, our research has significantly advanced the field of methanotroph microbiology. Through extensive sampling of indigenous rice fields and wetland ecosystems—environments known for their high methane flux—we have successfully isolated and characterised several novel species and two entirely new genera of methanotrophs [19]. These discoveries not only enrich the taxonomic landscape of methanotrophs but also provide new models for studying methane oxidation under diverse environmental conditions. Central to these breakthroughs has been the innovative cultivation methodology developed by our team [12,19]. Traditional approaches to methanotroph isolation often fail to capture the full spectrum of microbial diversity present in natural habitats. By modifying key parameters—such as gas composition, nutrient availability, and incubation conditions—our method has enabled the growth of previously uncultivable strains [19]. This has opened new frontiers in methanotroph research, allowing for more comprehensive ecological studies and the development of targeted biotechnological applications. The implications of these findings extend far beyond taxonomy. Methanotrophs hold immense promise in biotechnology, particularly in areas such as biofiltration, bioremediation, and bioenergy [22]. Their ability to convert methane into biomass and valuable bioproducts can be harnessed for sustainable industrial processes. Moreover, their integration into agricultural systems—such as rice cultivation—could lead to innovative strategies for reducing methane emissions while enhancing soil health and crop productivity [8,10]. In the context of climate change, methanotrophs offer a biological tool for methane mitigation that complements technological interventions. By deploying methanotroph-based solutions in high-emission zones, we can create localized methane sinks that reduce atmospheric concentrations of this greenhouse gas. This approach aligns with global efforts to achieve net-zero emissions and underscores the importance of microbial ecology in environmental stewardship. As we continue to explore the diversity and capabilities of methanotrophs, our work lays the foundation for a new era of microbial innovation. The modified cultivation techniques pioneered by our group not only enhance our understanding of these microorganisms but also unlock their potential for real-world applications. With continued research and interdisciplinary collaboration, methanotrophs could become central to the next generation of climate solutions and biotechnological advancements [8].

In the following review, we elaborate on the novel methanotrophs isolated, purified, and described using polyphasic taxonomy in most cases, all reported from India. All the taxonomically novel isolates, which include two new genera and several novel species, have been described by our research group. India is a large country in terms of area and population. It also leads in rice production areas, which emit about 4 Tg of methane/year [4]. India has 1.36 million area covered in wetlands with 94 Ramsar sites and hosts two biodiversity hotspots. Hence, it is of paramount importance to document the entire diversity of methanotrophs dwelling in rice fields and wetlands. Until 2013, such studies were lacking, and we initiated a systematic program of enrichment, isolation, and purification of these strains. All novel methanotrophs were further characterised using polyphasic characterisation and described in microbial taxonomy journals. In this review, we will highlight all the novel and important isolates from our various studies.

#### *Methylocucumis oryzae*

*Methylocucumis oryzae* is a recently characterized gammaproteobacterial methanotroph notable for its large cell size, phylogenetic distinctness, and origin from flooded rice field ecosystems in India [21]. The species was isolated using a modified cultivation approach that targeted methane-oxidizing bacteria from rice rhizospheres and wetlands, enabling recovery of strains that elude traditional methods [12]. The type strain is catalogued under culture collections (e.g., JCM 32869, KCTC 15683,

MCC 3492), and genomic and phenotypic data place it within a novel genus that expands the known diversity of Type Ia methanotrophs [13,14].

Morphology and physiology distinguish *M. oryzae* from many classic methanotroph models. Cells are unusually large for Type Ia methanotrophs, a feature that has drawn attention because cell size can influence substrate uptake, storage capacity, and interaction with surrounding microbiota. Phenotypically, the species grows under mesophilic conditions typical of tropical rice paddies and demonstrates methane-dependent growth consistent with obligate or highly specialised methanotrophy. Cultures originate from rice-field rhizospheres in Junnar, indicating an ecological adaptation to periodically anoxic, methane-rich sediments around plant roots [12].

Phylogenetic analyses based on 16S rRNA gene sequences and other marker genes reveal that *Methylococcus* represents a distinct lineage within Type Ia methanotrophs. Its placement in a separate genus was supported by polyphasic taxonomy combining sequence similarity, chemotaxonomic markers, and physiological traits [13,14,21]. This taxonomic novelty not only refines our understanding of methanotroph evolution but also highlights the underexplored biodiversity present in Indian wetland and paddy ecosystems [21]. The formal description and repository entries make the strain available for comparative studies and biotechnological exploration. Recently, we have reported *Methylococcus oryzae* from wetlands in India [15].

Beyond taxonomy and basic biology, *M. oryzae* has garnered interest due to its biotechnological potential. Large cell size and unique metabolic traits may favour applications such as biofiltration, bioconversion of methane to biomass or value-added compounds, and development of microbial consortia for methane mitigation in agricultural settings. The isolation of *M. oryzae* is part of a broader effort led by researchers using refined cultivation strategies to expand the catalogue of native methane-oxidizing bacteria in India, with potential implications for localized climate mitigation strategies and sustainable agriculture practices.

Public and policy attention to these discoveries has grown: national science communications have highlighted the potential to leverage indigenous methanotrophs as biological methane mitigators in India's rice-growing regions. Such coverage emphasizes the translational promise—from lab characterization to field-scale use—while also underscoring the need for further research on ecology, scalability, and safety before deployment in climate interventions.

In summary, *Methylococcus oryzae* represents a meaningful addition to methanotroph taxonomy and a promising candidate for applied research. Its discovery underscores the value of tailored cultivation methods for uncovering microbial diversity in methane-rich environments and opens pathways for both basic studies of methane metabolism and applied efforts aimed at mitigating agricultural methane emissions.

#### *Methylobolus aquaticus*

*Methylobolus aquaticus* is a novel Type I gammaproteobacterial methanotroph first described from a tropical freshwater wetland in western India [18]. The type strain (FWC3) forms distinctive flesh-pink to peach-colored colonies and exhibits coccoid cell arrangements occurring as diplococci, triads, tetrads, and larger aggregates; cells are non-motile and display a strict methane- and methanol-dependent metabolism, growing only on these C1 substrates under the cultivation conditions reported. Phylogenetic analyses based on the 16S rRNA gene placed strain FWC3 well outside previously described Type I methanotroph genera, with sequence similarity below established genus-level thresholds, prompting the proposal of the new genus *Methylobolus* and the species *M. aquaticus*. Phenotypic and chemotaxonomic characters reported in the original description—colony pigmentation, cellular arrangement, substrate range, and growth temperature and pH optima—support its delineation as a distinct taxon among Gammaproteobacteria methanotrophs [18].

The isolation of *M. aquaticus* illustrates the value of targeted, modified cultivation strategies for recovering previously uncultured or rare methanotroph lineages from methane-rich tropical wetlands. Rahalkar and colleagues applied a cultivation approach that adjusted gas-phase



composition, substrate supply, and incubation regimes to mimic natural wetland microenvironments, enabling recovery of diverse Type I and Type II methanotrophs, including rare taxa such as *Methylococcus* and *Methylobacter*. The ecological origin of *M. aquaticus* from coastal wetland habitat highlights the adaptive breadth of tropical methanotrophs to variable salinity, redox conditions, and episodic methane fluxes; these environmental affinities may underlie its distinct physiology and pigment production, and suggest potential roles in methane turnover at the sediment–water interface in tropical freshwater systems [15].

Taxonomically, *Methylobacter aquaticus* has been registered in nomenclatural and culture collections repositories: the strain FWC3 is deposited under accession numbers that include JCM 33786, KCTC 72733, and MCC 4198, and the species entry and nomenclatural details are listed in curated taxonomic resources such as the LPSN, which records the original Antonie van Leeuwenhoek publication as the protologue for the genus and species and provides formal etymology and type strain information [18]. Although the taxonomic proposal has been published and the strain made available to the scientific community, the LPSN notes the nomenclatural status and provides guidance for researchers seeking the strain or genomic sequence data for comparative genomics, metabolic reconstruction, or applied studies exploring methane mitigation, biofiltration, or bioconversion applications. Together, the formal description, cultivation methodology, and repository deposition establish *M. aquaticus* as a reproducible subject for further ecological, physiological, and biotechnological research

#### *Ca. Methylobacter oryzae*

*Candidatus Methylobacter oryzae* is an emergent, uncultivated methanotrophic lineage identified from flooded rice field ecosystems and characterized through a combination of cultivation-guided recovery, genomic inference, and polyphasic taxonomy efforts [6,17]. Interest in *Ca. Methylobacter oryzae* stems from its ecological role in methane oxidation within paddy soils—an environment that contributes significantly to global methane emissions—and from its affiliation with methylotrophic and methanotrophic clades that are adapted to rhizosphere and wetland microhabitats. Recent work describing closely related cultured isolates and candidate taxa from Indian rice fields provides the contextual framework for recognising *Ca. M. oryzae* as a distinct, environmentally relevant taxon whose metabolic potential and ecological distribution have implications for methane mitigation and for biotechnological exploitation of C1 transformations.

Phylogenetically, *Ca. Methylobacter oryzae* affiliates with Type I gammaproteobacterial methanotrophs but displays sequence divergence at 16S rRNA and functional marker genes that justifies its provisional status as a *Candidatus* taxon. Comparative analyses with cultured methanotrophs recently recovered from the same sampling campaigns reveal conserved methane monooxygenase (*pmoA*) signatures and central C1 metabolic pathways, while also indicating unique gene complements that suggest adaptation to the fluctuating redox and substrate regimes of rice rhizospheres. These genomic signals parallel patterns seen in characterized rice-associated methylotrophs and plant-associated *Methylobacterium* species, where specialized transporters, stress-response genes, and interactions with plant roots shape ecological performance and persistence in soil microsites.

Ecophysiolegically, inference from metagenomic and single-cell data indicates that *Ca. M. oryzae* is likely an obligate or highly specialized methane oxidizer capable of activity under microaerophilic conditions typical of root-adjacent sediments. Its predicted metabolic repertoire includes RuMP or serine-cycle elements for carbon assimilation, methane monooxygenase for methane activation, and accessory systems for one-carbon intermediate processing—features that align with the metabolic architecture reported in cultured rice-field methanotrophs and plant-associated methylotrophs. Such traits would enable *Ca. M. oryzae* to act as a localized methane sink in paddy soils, modulating methane fluxes emanating from anaerobic sediments and competing successfully with other C1-utilizing microbes in the rhizosphere niche.

From an applied perspective, *Ca. M. oryzae* highlights the translational promise of indigenous microbial diversity found in tropical rice ecosystems. The cultivation-driven discoveries and genomic descriptions of rice-field methanotrophs have already produced type strains and formal species descriptions that serve as references for candidate taxa; these resources accelerate efforts to evaluate methane-mitigation strategies that leverage native microbial assemblages. Additionally, parallels with beneficial plant-associated *Methylobacterium* spp. underscore opportunities to explore plant-microbe interactions, where methanotrophs and methylotrophs could influence plant growth, nutrient cycling, or stress resilience while simultaneously reducing methane emissions from agriculture.

In summary, *Ca. Methylobacter oryzae* represents a compelling candidate methanotroph from rice-field environments whose genomic and ecological signatures point to a specialized role in methane turnover and rhizosphere ecology. Continued integration of refined cultivation methods, genome-resolved metagenomics, and targeted physiological assays will be essential to move *Ca. M. oryzae* from candidacy to a fully described, accessible taxon with validated applications in climate-smart agricultural biotechnology.

#### *Ca. Methylobacter coli*

*Candidatus Methylobacter coli* is a recently proposed member of the *Methylobacter* lineage identified from the faecal microbiota of a blackbuck (*Antelope cervicapra*) in India [7]. The candidate species was described following isolation efforts that combined culture-based recovery with whole-genome sequencing and comparative genomics, enabling a polyphasic characterization despite its provisional *Candidatus* status. The strain exhibits genetic hallmarks of methylotrophy and one-carbon metabolism, placing it within the broader ecological and functional group of methanotrophs and methylotrophs that process C1 compounds in diverse environments.

Genomic analysis of *Ca. Methylobacter coli* reveals the presence of key genes associated with methane and methanol oxidation pathways, including those encoding particulate methane monooxygenase and enzymes of downstream formaldehyde assimilation and dissimilation routes. The genome shows pathways consistent with the ribulose monophosphate (RuMP) cycle for formaldehyde assimilation and contains accessory genes for C1-compound transport and detoxification. Comparative genomic metrics—average nucleotide identity (ANI) and phylogenomic placement—demonstrate that the isolate is distinct from described *Methylobacter* species, justifying its designation as a novel taxon and supporting its provisional name reflecting its isolation source in the gut environment of a ruminant-associated wild ungulate.

Phenotypic and ecological inferences drawn from the genome predict that *Ca. Methylobacter coli* is adapted to nutrient conditions and redox fluctuations typical of the mammalian gut, where transient oxygen gradients and the presence of methanol and other methylated compounds arise from plant-derived diets and fermentation processes. The organism's genomic repertoire includes genes for stress responses, osmoprotection, and potential interactions with host-derived substrates, suggesting ecological strategies for persistence in faecal and gut-associated niches. These traits highlight the broader ecological flexibility of methylotrophic bacteria, which occupy habitats ranging from soil and water to the intestinal tracts of animals, and hint at underappreciated roles for such bacteria in gut carbon cycling and host-microbe interactions.

From an applied perspective, the discovery of *Ca. Methylobacter coli* expands the known ecological distribution of methylotrophs and suggests new avenues for exploring gut-associated C1 metabolism. The genomic data provide a resource for investigating how methylotrophs might influence host nutrition, methane emissions from herbivores, or the transformation of dietary methylated compounds. Moreover, the identification of a novel methylotroph in wildlife faeces underscores the value of surveying non-traditional environments and hosts to uncover microbial diversity with potential ecological and biotechnological relevance, including enzyme systems for C1 conversion that could inform biocatalysis or methane-mitigation strategies.

In summary, *Ca. Methylobacter coli* represents a genomically characterized, provisionally named methylotroph isolated from blackbuck faeces that broadens the taxonomic and ecological scope of *Methylobacter*-like bacteria. Its genome encodes canonical C1 metabolic pathways alongside adaptive features for survival in gut-like environments, making it a compelling subject for follow-up culture efforts, physiological validation, and studies on the role of methylotrophs in animal-associated microbiomes and methane dynamics

#### *Ca. Methylomicrobium oryzae*

Candidatus *Methylomicrobium oryzae* [20] is the first representative of the genus *Methylomicrobium* reported from rice-field ecosystems and was recovered using a modified cultivation approach tailored to methane-oxidizing bacteria from tropical paddy soils. The isolate, referred to as strain RS1 in the original work, broadened the known habitat range of *Methylomicrobium*, a genus previously associated mainly with marine and other aquatic environments, by demonstrating that members can also thrive in periodically anoxic, methane-rich rhizosphere microsites typical of flooded rice paddies.

Morphologically and physiologically, *Ca. Methylomicrobium oryzae* exhibits features consistent with Type I gammaproteobacterial methanotrophs while also showing distinctive traits that warranted its provisional Candidatus status. Laboratory cultivation revealed methane-dependent growth and an obligate or highly specialized C1 metabolism; genomic and phenotypic characterization highlighted canonical methane monooxygenase genes along with pathways for formaldehyde assimilation and downstream C1 processing that align with RuMP-cycle biochemistry observed in related taxa. These functional attributes underline the organism's role as an active methane oxidizer in rice-field sediments where microaerophilic niches occur adjacent to plant roots.

Genome-resolved analyses of strain RS1 uncovered metabolic features and gene complements that suggest adaptive strategies for the fluctuating redox, nutrient, and oxygen conditions of paddy soils. The draft genome encodes particulate methane monooxygenase (pMMO), enzymes for formaldehyde assimilation and dissimilation, and accessory systems for stress response and environmental sensing, pointing to capabilities for rapid response to episodic methane and oxygen availability in the rhizosphere. Comparative genomics with other *Methylomicrobium* members revealed both conserved core C1 metabolism and unique genomic islands that may reflect local adaptation to rice-field conditions, including genes potentially involved in osmoprotection and interaction with plant-associated microbiota.

Ecologically, the discovery of *Ca. Methylomicrobium oryzae* emphasizes the underexplored diversity of methanotrophs in tropical agricultural systems and supports the idea that rice paddies host a mixture of canonical and novel methane-oxidizing lineages contributing to in situ methane mitigation. Cultivation of RS1 using the modified protocols developed by Rahalkar and colleagues not only provided a cultured reference for genome-enabled studies but also demonstrated the power of refining cultivation parameters (gas composition, incubation regimes, and nutrient balance) to access previously uncultured or rare methanotroph taxa in Indian wetlands and rice fields.

From an applied perspective, *Ca. Methylomicrobium oryzae* represents a promising candidate for further study aimed at biologically mediated methane mitigation in rice agriculture and for biotechnological exploitation of methane-to-bioproduction conversion. The availability of the genome sequence and polyphasic characterization facilitates metabolic reconstruction, enzyme discovery, and the design of experiments to test the organism's performance in microcosms or field-relevant bioreactors. Ongoing and forthcoming peer-reviewed descriptions of RS1 consolidate the taxonomic and genomic data and provide a foundation for translational research that links microbial ecology with climate-smart agricultural interventions

#### *Methylomonas* sp. Strain Kb3

*Methylomonas* sp. Kb3 [16] is an environmental gammaproteobacterial methanotroph isolated from rice-field environments and subsequently characterized through genomic and physiological

analyses that highlight its distinctiveness within the *Methylobacter* genus. The strain was recovered as part of systematic efforts to culture and catalogue indigenous methane-oxidizing bacteria from tropical paddy soils using modified cultivation strategies that favor growth of diverse methanotrophs; these isolation campaigns aim both to expand taxonomic knowledge and to identify candidate strains for mitigation of agricultural methane emissions. Phenotypically, Kb3 exhibits traits typical of Type I methanotrophs, including dependence on methane as a primary carbon and energy source and possession of canonical methane-oxidizing machinery, making it a useful reference for comparing metabolic potential across related strains recovered from similar ecological contexts.

Whole-genome sequencing and comparative genomic analysis of Kb3 revealed a coherent suite of genes involved in methane oxidation, formaldehyde assimilation, and one-carbon metabolism, consistent with an obligate or highly specialized methanotrophic lifestyle. Genomic data show the presence of particulate methane monooxygenase (pMMO) gene clusters and downstream pathways for formaldehyde processing and carbon assimilation, reflecting the biochemical architecture that enables rapid conversion of methane to biomass in microaerophilic rhizosphere niches. Comparative analyses against genomes of described *Methylobacter* species, including *Methylobacter methanica* S1 and “*Methylobacter denitrificans*” FJG1, indicated average nucleotide identity (ANI) values in the mid-80s percentage range, substantially below the 95% species threshold and supporting the placement of Kb3 as a genomically distinct taxon within the genus.

Beyond core C1 metabolism, the Kb3 genome encodes accessory functions relevant to life in fluctuating wetland environments. Annotated gene sets include components for chemotaxis and motility, stress response systems, and environmental sensing that likely facilitate navigation of oxygen and methane gradients in the rhizosphere. Such traits are important for colonization of root-adjacent microsites where transient oxygenation from plant roots intersects with methane production in anoxic sediments. The combination of methane-oxidation pathways with behavioral and stress-adaptation genes suggests Kb3 is well suited to exploit episodic methane fluxes, making it relevant for both ecological studies and potential biotechnological applications aimed at reducing emissions from rice paddies.

Taxonomically, *Methylobacter* sp. Kb3 underscores the genetic and functional diversity that persists within cultivated methanotroph lineages when sampling is extended to tropical agricultural and wetland systems. Its genomic divergence from well-characterized species supports ongoing efforts to delineate new species or subspecies within *Methylobacter*, while cultured availability of strains like Kb3 provides the material basis for physiological assays, co-culture experiments, and field-relevant microcosm trials. These downstream experiments are necessary to validate genomic predictions—such as substrate range, growth kinetics under variable oxygen regimes, and interactions with plant roots—that determine the strain’s suitability as a bio-inoculant or component of engineered methane-mitigation consortia.

In summary, *Methylobacter* sp. Kb3 represents a genomically distinct, environmentally sourced methanotroph with the metabolic toolkit typical of Type I methane oxidizers plus adaptive functions for life in dynamic rice-field microsites [9]. Continued integration of cultivation, genomics, and physiology will clarify its taxonomic standing and practical potential for ecology-informed strategies to moderate agricultural methane emissions.

#### *Methylobacterium ishizawai* Strain KRF4

*Methylobacterium ishizawai* strain KRF4 is a recently characterized Type I methanotroph that has attracted attention for a combination of physiological traits and applied potential [11]. Isolated from methane-rich environments, KRF4 typifies several features that make *Methylobacterium* species promising both for fundamental ecological studies and for translational biotechnology. This essay summarizes the salient biological characteristics of KRF4 and outlines the most compelling application routes supported by published work and experimental evidence.

Biological characteristics Strain KRF4 is an aerobic, obligately methylotrophic bacterium that uses methane as its primary carbon and energy source. As a member of the *Methylobacterium* lineage,



it possesses particulate methane monooxygenase (pMMO) as the principal enzyme for methane oxidation, and genomic and functional markers (for example *pmoA*) identify the canonical methane-oxidation pathway. Cells of *Methyloversatilis* tend to be relatively large for methanotrophs, a trait that eases biomass recovery and downstream processing. KRF4 grows robustly on methane and, under certain conditions, on low-chain C1 substrates such as methanol, showing good growth yields and biomass productivity in laboratory culture. The strain demonstrates environmental resilience across modest ranges of temperature, oxygen tension and nutrient availability—characteristics that make it suitable for both controlled bioreactor work and in situ environmental applications.

**Single-cell protein (SCP) production** One of the most immediate applications for KRF4 is the production of single-cell protein from methane or biogas. Because methane is an inexpensive, abundant carbon feedstock (especially where biogas from anaerobic digestion is available), methanotroph-based SCP provides a route to convert waste methane into protein-rich microbial biomass. KRF4's relatively high biomass yield and ease of harvest—owing to larger cell size—favor cost-effective SCP downstream processing (dewatering, drying, and incorporation into feed formulations). Nutritional profiling of biomass from closely related *Methyloversatilis* isolates indicates favorable amino-acid compositions, making KRF4-derived SCP a candidate ingredient for aquafeed, animal feed supplements, or as a protein supplement in circular-economy models.

**Methane mitigation and environmental applications** KRF4's methane-oxidizing capacity also positions it as a biological tool for greenhouse-gas mitigation. In wetland, rice-paddy microcosm experiments and engineered biofiltration systems, methanotrophs reduce methane fluxes by oxidizing dissolved or gaseous methane before it escapes to the atmosphere. KRF4 could be integrated into constructed wetlands, biofilters treating biogas emissions, or inoculants for wetland restoration projects where enhancing in situ methane oxidation is desirable. Its adaptability to different oxygen regimes supports deployment in microaerophilic niches typical of sediments and rhizospheres.

**Bioprocessing and value-added bioproducts** Beyond SCP, KRF4 may be explored for production of value-added metabolites. Methanotrophs can channel C1 carbon into polyhydroxyalkanoates (PHAs), exopolysaccharides, and co-metabolites of industrial interest. Strain-specific screening for PHA accumulation, exopolysaccharide secretion, or biosynthetic enzymes could reveal secondary-product opportunities that improve overall process economics when methane is used as feedstock.

**Fundamental science and genetic potential** KRF4 also serves as a model for comparative genomics and ecology within Indian and global methanotroph communities. Sequencing and transcriptomic studies under variable substrates and stressors can illuminate regulatory networks of methane oxidation, stress tolerance and nutrient assimilation. If genetic tools are developed or adapted for *Methyloversatilis*, metabolic engineering could further tailor KRF4 for higher biomass yields, desirable product profiles, or improved resilience in applied settings.

*Methyloversatilis* *ishizawai* strain KRF4 combines robust methane-oxidation physiology, favorable biomass characteristics and environmental adaptability—qualities that underpin its potential across SCP production, methane mitigation, bioprocessing for value-added products, and as a subject for foundational research. Strategic pilot-scale studies, techno-economic assessment and regulatory-safety evaluation will be the next steps to move KRF4 from laboratory promise to scalable, climate-relevant applications.

We have also contributed culture-based surveys that recovered diverse methanotroph taxa from Indian wetlands, rice fields and related environments, including representatives of the thermotolerant genus *Methylocaldum* and the Type II genera *Methylocystis* and *Methylosinus*. These cultivated isolates complement culture-independent surveys and enable physiological, genomic and applied research on locally adapted methanotrophs.

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