Methanotrophs: The Natural Way to Tackle Greenhouse Effect

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Abstract

Methane is considered as an important greenhouse gas which produced from a wide range of anthropogenic and natural sources. It plays a key role in global warming. Capturing and disposal of methane is technically both costly and problematic. A low-cost alternate to the conventional methods is the microbial oxidation of methane. Methanotrophs are the type of bacteria that aerobically oxidize methane as a source of energy through their key enzyme, mono-oxygenase (MMO), especially the soluble MMO, it is noteworthy in its broad substrate specificity. This exceptional capability, i.e., catalyzing reactions of environmental importance, has earned methanotrophs attention from applied microbiologists and biotechnologists. From about 30 years, it is observed that copper (Cu) is playing an important role in the physiology and activity of methanotrophs, but the discovery of how Cu collect by these cells, more importantly what is the role Cu playing in CH4 oxidation by the particulate CH4 mono-oxygenase and how Cu affects the ability of methanotrophs to oxidize different substrates has been made. In this review we summarize the morphology, phylogeny, ecology, and possible applications of methanotrophs to address the global as well as regional issues, along with the role of gene expression regulation by Cu and how it affects the cell activity of methane-oxidizing bacteria. Our focus was on two main aspects of potential value and application of methanotrophs in environmental bioremediation, namely physiology along with working of methanotrophs and methane removal from atmosphere.

Keywords: Methanotrophs; Mono-oxygenase (MMO); Methane; Greenhouse effect; Methanobactins

Introduction

Methane (CH4) is a potent greenhouse gas and its concentration in the atmosphere is since forever on an up rise with an approximate 1% increase per year [1]. It is a major contributor to direct radiative forcing from all the long lasting greenhouse gases (GHGs) accountable for about 20% of it (IPCC, 2001). Industrial revolution has led to the amplified CH4 concentration from 0.7 to 1.8 p.p.m.v. due to increased anthropogenic inputs [2]. This increased anthropogenic emission of greenhouse gases is what has led to the globally hyped phenomena known as “The Greenhouse Effect”. This occurrence can potentially direct towards hazardous changes in climate namely temperature fluctuations leading to disastrous outcomes.

GHGs, chiefly CH4 reduction can lead to stabilization of the effect resulting in optimization of global temperatures. However, curbing these gases is difficult i.e., capturing and disposal of methane is technically costly and problematic. A low-cost alternate to the conventional methods is the microbial oxidation of methane [3,4].

Methanotrophs aka Methane-oxidizing bacteria, are a major group of bacteria primarily responsible for running the naturally present global methane cycle. Thus, methanotrophs consist of one of the major groups of free-living microorganisms that metabolically playing role in determining the nature of the environment. Although it is self-evident that methanotrophs play a role in maintaining low levels of methane in the atmosphere but to do so they require certain conditions and environmental factors [5].

Phylogenetic and physiological diversity of methanotrophs should be considered of prime focus while working with them since they are newly discovered vague assets of bioremediation at the moment. Some other important features to be considered are how methanotrophs can be used as a pollutant degrader by removing greenhouse gas and Cu role in methanotrophic regulating activities by shedding light on mechanism for its uptake used by methanotrophs [6].

The above mentioned aspect are examined in this review, and an attempt is made to give a contemporary view of the environmental significance of these microorganisms and to evaluate the potential for their industrial exploitation.

Diversity of Methanogens: A Taxonomic and Phylogenetic overview

Methanotrophic bacteria have been a center of attention since their discovery by Kaserer and Søhngen in the start of 20th century. In 1906, *Bacillus methanicum* was the first methane oxidizing bacteria to be isolated but it was not until Whittenbury and his colleague's isolation and characterization of over a 100 new methanotrophs that the current basis of classification of these bacteria was established [7].

Classification

The classification of methanotrophs according to researchers is as so that these bacteria are separated into five groups (proposed genera) on the basis of resting stage formed, morphological differences, structure of intracytoplasmic membranes and some other physiological characteristics. Study shows that, methanotrophs have 14 recognize genera, which then categorized into 3 main types i.e., type I, type II and type X, based on the above mentioned basis i.e., morphology,
structure of membrane and some other physiological characteristics (Figure 1). The discovery of diverse types helped us to further study of methane-utilizing bacteria and has led to the belief that natural environment is more suitable for them [5,7].

**Types**: Type I is the type of methanotrophs that dominates in environments where methane is present in a limited quantity and relatively high levels of combined nitrogen and copper are present. Type II bacteria on the other hand favors environment with high concentration of methane, as well as the environment where dissolved oxygen level is low, and restrictive combined concentration of nitrogen and/or copper. Type I strains can also be characterized on the basis of having other apart from condition characteristics i.e., they have intracytoplasmic membranes throughout the cell in the form vascular disk present in bundles, for carbon absorption they use ribulose monophosphate (RuMP) pathway. They also have phospholipid fatty acids which consist of carbon length of 14 and 16.

**Type X**: The best of both strains: Type X strains are a combination of Type I and II strains i.e., they have phospholipid fatty acids of 16 carbon, a RuMP pathway along with possessing ribulose-1,5-bisphosphate, and ability to grow at a temperatures which is higher than Type I or II strains [5]. Type X methanotrophs though are quite similar to Type I they are distinguished from type I methanotrophs on the basis that they have enzyme of serine pathway which are lower in levels named as ribulosebisphosphate carboxylase. This enzyme is present in the Calvin Benson cycle. Type X have a DNA, with a distinctive property that it contains G+C content with high moles percentage as compared to type I methanotrophs. Type X have the property that they can grow at higher temperature which isn't present in any other type [10,11].

**Proteobacteria defined types**: The main difference between the types is the pathway they utilize. For the assimilation of carbon type I methanotrophs utilize RuMP pathway, as they are Gammaproteobacteria (Figure 2). As type II methanotrophs uses Serine pathway for carbon absorption, so they are Alphaproteobacteria [12].

**Ecology**

Majority of methane-oxidizing species of bacteria isolated from a wide range of environments suggest that they are mostly aerobic and obligate in nature. However, it cannot be established as a fact due to two main reasons. Firstly, for a fact methane is continuously generated in anaerobic environments, and there is good evidence for its anaerobic oxidation linked which is linked to sulfate reduction by uncharacterized microorganisms present in sediments. Secondly, there is no surety as to if this is the true reflection of relative abundance or just an artifact due to isolation procedures. A thing for ecology for sure is that aerobic environment e.g., soils, surface layers of sediments and natural waters where methane is diffusing, have diversity in aerobic methanotrophs population [13,14].

**MMO at Work**

Methane monoxygenase (MMO), all known aerobic methanotrophs the first step in oxidation is the methane conversion into methanol by using MMO [15]. In the second step the methanol further oxidized into formaldehyde, after that it has two options, first is to convert into biomass and other is the further oxidation into formate and then into CO₂.

Two iso-enzymes of MMO is known: soluble MMO (sMMO), found only as subset of known methanotrophs, and also membrane bound (or particulate) MMO (pMMO). It is located in specialized internal membrane structures, called ICMs [15,16]. sMMO and pMMO both have mixed function of oxidation, that is one atom from O₂ goes to methanol and the other to water, involving the input of 2 electrons and 2 protons. sMMO utilizes NADH, but it is still unknown that what is the physiological electron donor to the MMO [15].

**pMMO vs sMMO**

pMMO is found in almost all known methanotrophs, it also shows more affinity towards methane when it is compared to cells which are expressing sMMO (Figure 3). Further studies show that cells shows
higher growth yield which are using pMMO for growth, which signifies that oxidation of methane is more effectual by pMMO [17].

**Figure 3**: A flowchart of methanotrophs and methanotrophic metabolism for utilizing methane.

M Ethanotroph “The Degrader”

In the prevailing twenty-five years, study shows that methane-oxidizing bacteria have the ability to degrade wide ranges of halogenated hydrocarbons. Methanotrophic enrichments are capable of degrading priority pollutants e.g., chlorinated hydrocarbons [18], in US and various other countries they are present in aquifers, landfills, wastewaters, and waste disposal sites [19,20]. Methanotrophs make for a good pollutant degrader given the abilities that methanotrophs are ubiquitous and the rate of degradation of pollutant with the help of methanotrophs have magnitude greater than that have witnessed by other cells having monooxygenases [21].

pMMO: The main degrader MMO

sMMO was initially thought of as the MMO responsible for degradation given it’s broader substrate range. However, in a study by DiSpirito et al. [22], it is stated that pMMO could indeed degrade trichloroethylene shattering the old belief that it cannot degrade chlorinated solvents [22]. On a side note it was noted that increasing Cu availability led to increased degradation of trichloroethylene [23,24].

Primarily owing to mRNA levels and enzymatic activity which is present in different environments, it is largely difficult to measure prevalent form of MMO expressed in *in-situ* communities [25]. Using competitive RT-PCR assays cultures from land resources like soil slurries and groundwater from a mixture of two gases i.e., chloroethane/chloroethene plume were observed they showed only pMMO instead of sMMO concluding that on the site degradation of chlorinated hydrocarbons are done by the pMMO expressing cells [26].

Further lab studies show that survival chance of pMMO expressing cells is increases if chlorinated ethenes are present and also they have the ability of degradation of these compounds in higher concentration because of pMMO specificity of CH₄ and pollutant transformation to more toxic products in slow time [27,28] (Figure 4).

**Unique Cu-Specific Uptake Systems Present in Methanotrophs**

Copper is considered to be the key factor in controlling Methanotrophic activity along with being of central importance in the physiology and metabolism of methanotrophs. From past decade the activity of methane-oxidizing bacteria is popular, particularly from the perspective of sMMO and pMMO activities [29-31]. Methanotrophs respond differently toward the diverse concentration of Cu. Organic acids such as humic acid can decreased the bioavailability of Cu especially in subsurface environments leading to different response of methanotrophs [32].

Copper is important because pMMO is copper dependent i.e., the first step in methanotrophic metabolism pathway depends on it, while on the other hand copper determines the MMO type that is during copper starved conditions sMMO are produced. Few methanotrophs strain possess copper mediated switches between MMO’s helping in transcription repression of sMMO gene, which resulting in the enhancing of pMMO expression, as well as formation of the intracytoplasmic membrane. This copper switch mechanism is yet to be clarified [15,33].

**Methanobactins: The copper binding molecules**

Recent studies have suggested that Methanobactin (Mb) plays important role in copper uptake by methanotrophs. They are basically copper-binding molecules with low-molecular mass, and are secreted by many methanotrophic bacteria, specifically *M. trichosporum* OB3b. Methanotrophs fulfill their copper requirement by the secretion of mb. They bind either Cu⁺ without changing their oxidation state or Cu²⁺ which is later reduced to Cu⁺ [34,35]. Choi et al. [36] suggested that Cu-mb work as a vehicle which directs the electrons from the *in vivo* reductant toward the dinuclear Cu site, with subsequent transfer to the diiron site for CH₄ oxidation [36] (Figure 5).
Application of Methanotrophs in Greenhouse Gas Removal

Global warming poses a fundamental threat to planet Earth, and emission of greenhouse gases play a key role in this. From the decade questions like: What's the best way to reduce anthropogenic emission of these gases? How CH$_4$ emitting from landfills and agricultural soil can be prevented? Have been asked.

CH$_4$ sink

Approximately 10-90% of CH$_4$ produced from the methanogenic activity is consumed by methanotrophs even before it can be released into the environment, making methanotrophs key proprietor in degradation of CH$_4$ from atmosphere for e.g., acidic forest soil express pMMO activity for Methanotrophic oxidation of CH$_4$. Reduction of free CH$_4$ with the help of methanotrophs is now attracting attention from the researchers since this activity broadly occurs in landfills which covers soil thus making landfills natural CH$_4$ sinks [37-39].

Engineered strategies for methane removal

Various strategies can be adopted considering stimulation of Methanotrophic activity for CH$_4$ reduction in atmosphere. 'Biofilters or Biocovers' are the well-known engineered system involved in this activity (Figure 6).

Biocovers are typically organic matter, where the permeable material, may it be compost, sewage sludge or wood chips, cover the surface area of landfill. These materials have the characteristic of effective gas transportation for both CH$_4$ coming out from landfills and the atmospheric oxygen, along with the ability of water retention for the Methanotrophic activity [40,41].

Strain discovery (pMMO2)

Knief and Dunfield isolated two strains of methanotrophs which helps in CH$_4$ reduction by the time period of 3 months, these strains however are unable to grow in atmospheric concentration of CH$_4$ and require special conditions. Surprisingly, a new strain of Methylocystis has been discovered which has the ability to grow at 10 p.p.m.v. CH$_4$. Further studies of this strain shows that this cell has two pMMO isozymes, pMMO1 and pMMO2 leading to the discovery that Oxidation of CH$_4$ by pMMO2 is more effective than pMMO1 [42,43].

Biotechnology Meets Methanotrophs

There are only few methane based products present commercially but, none of them involves methanotrophs strains which are metabolically engineered. The reason behind this is the limited study of methanotrophs and studies only being done during the production process for biopolymers, vitamins, antibiotics, single cell protein (SCP) as well as carboxylic acids [16,44].

However, the studies that have been done show that poly-β-hydroxybutyrate (PHB), which is a biopolymer produced by methanotrophs have biomedical applications, can be used as biodegradable food packaging, and encapsulation of agrochemicals on a large scale [45].

Biotechnology play an important role in methanotrophic bioremediation by the conversion of methane into SCP at industrial scale by Methanotrophic cultivation. This concept is not more than 3 decades old, but remarkably enhancement is only now in rapid expansion of genetic and metabolic knowledge [46,47].

Unfortunately limited studies are done to make methanotrophs useful for the purpose of biocatalysts in the lipid or fuel production with Bio-GTL process, mainly because there is no suitable strain is produced or discovered [48].

Scientists of 21st century are now using biotechnology for enhancing Methanotrophic bacteria effectively, that is controlling of methane emission, bioremediation, biobleaching and well known Methanotrophic based biofilters for landfill methane reduction [41,49].

MB an asset for biotechnologist

Many species of methanotrophs, which expresses pMMO activities, produce Methanobactin (mb), when there is high demand of copper, as...
it binds and reduce copper at high affinity. This high binding affinity catch interest of biotechnologists, for using them in biotechnological application including controlling of copper homeostasis in Wilson's disease patient by working as a therapeutic agent [50].

Interestingly, mb can also bind other metals like binding and reduction of both trivalent gold and bivalent mercury ions, their mechanism of binding is very much similar to that of copper ion binding. This can lead to the using of methanotrophs in the field of biobleaching with mining and environmental remediation. Gold nanoparticles and uniform copper can also be produced by Methanobactin [36,49].

Conclusions and Future Prospects

In this review, we try our best to summarize current knowledge on morphology and application of methanotrophs in environmental bioremediation, mainly for removal of methane from atmosphere. These interesting microorganisms were merely discovered not more than a century ago and attracting great interest. There are plenty more possibilities then discussed in the literature above to make methane-oxidizing bacteria become important and universal microorganism industries. Role of methanotrophs in biogeochemical carbon cycle and also in controlling of global climate change can’t be neglected. There is still too much to discover about these organisms like how can unique Methanotrophic structures such as acidophilic, thermoacidophilic, and nitrite-utilizing methanotrophs be best used for the purpose of pollutant degradation? How prevalent are these types of methanotrophs? Can they be with little effort stimulated in situ? Answers to all the above issues will not only guides us to the use of methanotrophs for pollutant degradation but will also help act as a gateway to other interesting issues in methanotrophy. We believe that mighty progress in basic research, together with novel and cutting-edge biotechnological methods eventually will enable the engineering applications of methanotrophs to be realized.

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