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REVIEW

Methanotrophs: promising bacteria for environmental remediation

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Abstract Methanotrophs are unique and ubiquitous bacteria that utilize methane as a sole source of carbon and energy from the atmosphere. Besides, methanotrophs may also be targeted for bioremediation of diverse type of heavy metals and organic pollutants owing to the presence of broad-spectrum methane monooxygenases enzyme. They are highly specialized group of aerobic bacteria and have a unique capacity for oxidation of certain types of organic pollutants like alkanes, aromatics, halogenated alkenes, etc. Oxidation reactions are initiated by methane monooxygenases enzyme, which can be expressed by methanotrophs in the absence of copper. The present article describes briefly the concerns regarding the unusual reactivity and broad substrate profiles of methane monooxygenases, which indicate many potential applications in bioremediation of heavy metals and toxic organic compounds. Research is needed to develop understanding in plant–methanotrophs interactions that optimize methanotrophs utilization in the field of environmental remediation, while supporting other ecosystem services.

Keywords Bioremediation · Heavy metals · Methanotrophs · Organic pollutants

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Introduction

There is a growing concern about global warming worldwide. Methane (CH_4) is one of the greenhouse gases (GHGs), which contributes to global warming. Methane is about 23 times more effective as a greenhouse gas than carbon dioxide (CO_2) (Hasin et al. 2010). Methanotrophs are the only known significant biological sink for atmospheric CH_4 and play a crucial role in reducing CH_4 load up to 15 % to the total global CH_4 destruction (Singh et al. 2010). Methanotrophs exist in a variety of habitats due to having physiologically versatile nature and found in a wide range of pH, temperature, oxygen concentrations, salinity, heavy metal concentrations, and radiation (Barcena et al. 2010; Dubey 2005; Durisch-Kaiser et al. 2005; Lindner et al. 2007; Tsubota et al. 2005). Broad-spectrum methane-oxidizing methane monooxygenase (MMO) enzyme is found only in methanotrophs, which possess two forms, namely membrane-associated or particulate form (pMMO) and soluble or cytoplasmic form (sMMO). The pMMO is found in all known methanotrophs except for the genus *Methylocella* (acidophilic) (Theisen et al. 2005), while the sMMO is present only in a few methanotroph strains (Murrell et al. 2000a, b).

There are several sources that generate huge amount of toxic heavy metals/metalloids (Cr, Cd, Pb, As, Cu, Zn, Ni, Hg, etc.) and organic pollutants into the environment (Wijnhoven et al. 2007). Soils contaminated with heavy metals and/or organic pollutants are generally left abandoned for several years and therefore may not be safe for agricultural production. Recently, microbial bioremediation techniques have been found to alleviate the metal/organic pollutant toxicity of contaminated soils (Hasin et al. 2010; Shukla et al. 2009). Methanotrophs may be promising bacteria for environmental bioremediation

(Jiang et al. 2010). Methanotrophs have been shown to degrade/co-oxidize diverse type of heavy metals and organic pollutants due to the presence of broad-spectrum MMO (Lindner et al. 2005; McFarland et al. 1992; Smith and Dalton 2004). The MMO has been shown to oxidize a wide range of substrates, including aromatic compounds viz. halogenated benzenes, toluene, and styrene as well as aliphatic hydrocarbons with up to eight carbons (Burrows et al. 1984; Colby et al. 1977; Green and Dalton 1989).

Some of the comprehensive review articles provide basic status and different perspectives of methanotrophs viz. research history (Dalton 2005), extremophilic methanotrophs (Trotsenko and Khmelenina 2002), taxonomy and ecology (Hanson and Hanson 1996), methanotrophs and CH_4 oxidation related to wetlands (Chowdhury and Dick 2013), properties of methane monooxygenase (MMO) (Lieberman and Rosenzweig 2005), metabolic aspects (Trotsenko and Murrell 2008), biochemistry (Anthony 1982; Hakemian and Rosenzweig 2007), gene regulation (Murrell et al. 2000a, b), biotechnological applications (Dalton 2005; Trotsenko and Khmelenina 2005), molecular marker (Dumont and Murrell 2005; McDonald et al. 2007). None of them paid attention to explain about bioremediation potential of methanotrophs. So, there is an urgent need to re-tabulate the real involvement and benefits of this unique microbe in bioremediation of toxic pollutants. Broad substrate profiles of MMOs and its unusual reactivity indicate diverse potential applications of methanotrophs in bioremediation. Advancement in our knowledge about methanotrophic bioremediation may facilitate their wide applications for safe and sustainable environmental development. This article has been aimed to highlight the bioremediation potential of methanotrophs and provides a moderate review on the progress made in methanotrophic research related to bioremediation and identifying the critical research needs for developing and implementing successful methanotrophic bioremediation as a model worldwide. The possible ways to maximize its multiple uses for mitigating the various pollutants are also suggested.

Remediation of hazardous organic pollutants by methanotrophs

Methanotrophs synthesize both particulate and soluble forms of methane monooxygenases (pMMO and sMMO, respectively), which can co-metabolize diverse type of hydrocarbons and halogenated organic compounds including aromatics. The priority pollutants like trichloroethylene (TCE) can be easily degraded by application of methanotrophs (Jiang et al. 2010; Kikuchi et al. 2002; Shukla et al. 2009). The majority of methanotrophs are known to produce particulate methane monooxygenase (pMMO) except few strains like *Methylcella palustris* (Dedysh et al. 2000)—a known producer of soluble methane monooxygenase (sMMO). The sMMO-expressing methanotrophs, due to their relatively broad substrate range (Shigematsu et al. 1999) and fast turnover kinetics, exhibit fast decline in the level of pollutants than that calculated for pMMO-expressing methanotrophs. In contrast to pMMO, which works on a very narrow spectrum of carbon substrate (alkanes and alkenes), the sMMO is capable of oxidizing a wider range of organic compounds including aliphatic, aromatic hydrocarbons, and their halogenated derivatives (Trotsenko and Murrell 2008).

In contrast to other microbes that are recognized to degrade halogenated hydrocarbons via reductive pathways (Maymo-Gatell et al. 1999), the biodegradation of chlorinated hydrocarbons by methanotrophs occurs in an oxidative manner (Lontoh et al. 2000). The oxidative biodegradation carried out by MMO appears more significant than the reductive dechlorination of chlorinated ethenes, such as TCE and tetrachloroethylene, which often results into accumulation of more toxic intermediates, e.g., vinyl chloride, a known potent carcinogen (Maymo-Gatell et al. 1999). On the contrary, the MMO-mediated oxidative mechanisms of degradation of halogenated compounds by the methanotrophs do not accumulate hazardous intermediates (McCue et al. 2002). Thus, the applicability of methanotrophic degradation of halogenated hydrocarbons for in situ bioremediation of contaminated soil and water system can be a safer remediation technique for sustainable development of the environment.

Earlier it has been reported that trichloroethylene (haloalkenes), a volatile chlorinated organic pollutant, is generally resistant to biodegradation by microorganisms (Wilson and Wilson 1985), but methanotrophs have been shown to co-metabolize trichloroethylene by the potent MMO enzyme (Van Hylckama Vlieg and Janssen 2001). Stockholm Convention banned the use of persistent organic pollutants (POPs) like lindane, which are highly carcinogenic, persistent, bio-accumulative and endocrine disruptor (ATSDR 2005). Under aerobic conditions, the degradation of lindane [γ-hexachlorocyclohexane ($C_6H_6Cl_6$)] by bacteria occurs through repeated steps of dehydrochlorination and dechlorination, and it gets converted to chlorobenzenes and the end-product is carbon dioxide, which can be taken up by plants (Mathur and Saha 1975; Vonk and Quirijns 1979). New, low cost and rapid screening tool for the detection of microbes capable of degrading lindane has been discovered (Phillips et al. 2001). The same assay

technique may be used for identifying lindane-degrading methanotrophs. Thus, there is an urgent need to examine degradation of lindane by the potent MMO enzyme of the methanotrophs. Similarly, the methanotrophs-mediated environmental fate and degradation of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) need to be explored. Biodegradation of potentially toxic PAHs has been examined by non-specific MMO enzyme of marine methanotrophs (Rockne et al. 1998). The aerobic catabolism of a PAH molecule by bacteria occurs via oxidation of PAHs to a dihydrodiol by a multicomponent enzyme system. The mechanism of bioremediation of PAH involves *ortho* cleavage or *meta* cleavage type pathway, resulting in the formation of protocatechuates and catechols that are then converted to tricarboxylic acid cyclic intermediate (Kanaly and Harayama 2000). In the biodegradation of PCBs, the ability of methanotrophic bacterium *Methylosinus trichosporium* OB3b, expressing soluble methane monooxygenase, has been shown to oxidize a range of *ortho*-halogenated biphenyls (2-chloro-, 2-bromo-, and 2-iodobiphenyl) although at lower rates than the unsubstituted biphenyl (Lindner et al. 2000). Hydroxylation is the dominant reaction during the degradation of the aromatic ring, followed by dehalogenation. This type of study provides a platform on how methanotrophs can be used in conjunction with anaerobic degraders for the removal of highly chlorinated biphenyls. Additional research is still needed to determine how the products of methanotrophic oxidation of *ortho*-substituted biphenyls are further oxidized by heterotrophic microorganisms to ensure complete mineralization (Lindner et al. 2000). Some of the reports emphasize that polar regions are interesting because of the presence of POPs, transported by a complex mechanism involving successive volatilization and deposition steps from warmer areas toward cooler regions including Antarctica (Bengtson 2011). Barcena et al. (2010) have reported methanotrophic methane oxidation in Antarctica. There are several reports about the existence of different psychrophilic methanotrophs from Antarctica (Bengtson 2011). But very little information is available about the nature of psychrophilic methanotrophs diversity in contaminated sites, the genes that confer them the capability for bioremediation as well as survival in the extreme low temperature.

Pentachlorophenol (PCP), a highly substituted aromatic compound, is extensively used as wood preservative, bactericide, fungicide, and herbicide (Yuancai et al. 2007). PCP has been listed as a pollutant by the US Environmental Protection Agency owing to its toxicity. Under aerobic condition, PCP is first converted to tetrachloro-p-hydroquinone by hydroxylation, and daughter products are trichlorohydroquinone and dichlorohydroquinone, because the activity of monooxygenase and dioxygenase was inhibited by the substituted chlorine (Rasul and Chapalamadugu 1991). In anaerobic condition, the PCP is first reductively dechlorinated and converted to *tetra*-, *tri*-, *di*-, and mono-chlorophenol (TetCP, TCP, DCP, MCP). Nevertheless, these intermediates are more toxic than PCP and are difficult to degrade (Yuancai et al. 2007). Since aerobic degradation of metabolites is more feasible, complete degradation of PCP is possible with the use of aerobic methanotrophs and anaerobic microorganisms. Gerritse et al. (1995) and Tartakovsky et al. (1998) have been able to demonstrate complete degradation of tetrachloroethylene (aromatic compound) by using anaerobic dechlorinating and aerobic methanotrophic enrichment cultures or a consortium of co-immobilized methanogenic and methanotrophic bacteria, respectively (Tables 1, 2).

Remediation of heavy metals by methanotrophs

Methanotrophic bacteria have considerable potential for use in biotechnology and in bioremediation due to the amenability of these bacteria to large-scale cultivation (Jiang et al. 2010; Overland et al. 2010; Semrau et al. 2010). It has been suggested that methanotrophs also influence the speciation and bioavailability of metals in the environment (Choi et al. 2006; Jenkins et al. 1994). Hasin et al. (2010) have reported the reductive transformation of soluble and more toxic Cr(VI) into a less toxic Cr(III)species by methanotrophic bacteria (*Methylococcus capsulatus* Bath), as the Cr(III) is insoluble and tends to get precipitated at high pH.

The importance of a toxicity reducing, Cu-carrier molecule is mainly linked to methanotrophs given their typical habitat, i.e., geochemically distinct microaerophilic zones. In such sites, intense redox cycling leads to active precipitation of Mn and Fe oxides (Ferris et al. 1999). CH_4 oxidation requires Cu (due to its high reactivity), which, in turn, demands a strong Cu defense system. There is a molecular carrier for Cu, termed as methanobactin (mb)—a 1,216-Da fluorescent metal-binding chromopeptide (Kim et al. 2004), which confers protection to the cells both from external and internal Cu toxicity. The study of Knapp et al. (2007) provides a strong evidence about the mb-mediated Cu release from the mineral stage, which changes the Cu availability and allows pMMO gene expression in methanotrophs. Therefore, mb might be particularly critical for ecological success of methanotrophs in such metal-polluted environments where proteins like methanobactin (mb) allow the selective acquisition of Cu, while protecting the

Table 1 Methanotrophic bacteria involved in the bioremediation of various toxic hydrocarbon and heavy metal pollutants

Methanotrophic species	Targeted organic pollutants	References
Methylosinus trichosporium OB3b	Halogenated hydrocarbons	Hanson et al. (1990), Oldenhuis et al. (1991)
Methylomonas albus BG8, *Methylocystis parvus* OBBP, and *Methylosinus trichosporium* OB3b	Polynuclear aromatic hydrocarbons and transition metals	Jenkins et al. (1994)
Methylosinus trichosporium OB3b	TCE	Lontoh and Semrau (1998)
Type II methanotrophs	Phenanthrene, anthracene, and fluorene	Rockne et al. (1998)
Methylocystis sp. M, *Methylococcus capsulatus* (Bath), *Methylosinus trichosporium* OB3b, *Methylosinus sporium* strain 5, and unidentified strains of methanotrophs (MP18, MP20, P14)	TCE-degradation	Kikuchi et al. (2002)
Type II methanotrophs	TCE	Shukla et al. (2009)
Methylosinus trichosporium OB3b and *Methylocystis daltona* SB2	TCE, DCE, and VC	Yoon (2010)
Methylocystis strain SB2	Vinyl chloride (VC), dichloroethylene (DCE), trichloroethylene (TCE), and chloroform (CF)	Im and Semrau (2011)
Methanotrophic mixed culture	Biotransformation of three hydrochlorofluorocarbons (HCFCs) and one hydrofluorocarbon (HFC)	Chang and Criddle (1995)

Methanotrophic species	Targeted inorganic pollutants	References
Methylophilus methylotrophus EHg7	Cadmium (Cd)	De Marco et al. (2004)
Methylophilus methylotrophus ECr4	Chromium (Cr)	De Marco et al. (2004)
Methylococcus capsulatus Bath	Chromium (Cr)	Hasin et al. (2010)

Table 2 Methanotrophs that have been isolated from a wide variety of habitats/environments

Variety of habitats	References
Soils	Dubey (2005)
Sediments	Tavormina et al. (2008)
Landfills	Ait-Benichou et al. (2009)
Groundwater	Lindner et al. (2007)
Seawater	Durisch-Kaiser et al. (2005)
Peat bogs/peat lands	Larmola et al. (2010)
Hotsprings	Tsubota et al. (2005)
Plant rhizosphere	Qiu et al. (2008)
Salt reservoirs	Heyer et al. (2005)
Antarctic	Barcena et al. (2010)

methanotrophs against other similar potentially toxic metals. Microbial-based bioremediation of heavy metals, produced from metal plating, tanning, paper-making industries (Cervantes et al. 2001; Hasin et al. 2010; Zayed and Terry 2003), can be used for detoxification of metals through their conversion to less toxic and less soluble form like Cr(III). Hasin et al. (2010) reported a well characterized model of methanotroph *Methylococcus capsulatus* (Bath), capable of bioremediation of chromium (VI) pollution over a wide range of concentrations (1.4–1,000 mg L^{-1} of Cr^{6+}). The genome sequence of *M. capsulatus* (Bath) suggested at least five genes for the chromium (VI) reductase activity in this bacterium. Study of De Marco et al. (2004) was the first attempt to systematically analyze the capability of methylotrophic strains to develop tolerance against heavy metal pollutants. These workers isolated thirty-one novel methylotrophic bacterial strains from a range of soil and sediment sources (both pristine and polluted). Furthermore, they noted that some of the isolates exhibited interesting characteristics of resistance to heavy metals, arsenate, or organic pollutants. Among them, four strains were considered as real 'super-bugs' for their ability to withstand extremely high concentrations of a variety of heavy metal pollutants. The toxic mercury (II) ion is usually detoxified by bacteria via its reduction to elemental mercury, catalyzed by an NAD(P)H-dependent mercuric reductase enzyme (EC 1.16.1.1). It has been proved that *Methylococcus capsulatus* (Bath)—a methanotrophic member of the *Gammaproteobacteria*—uses this enzyme to detoxify mercury (De Marco et al. 2004). In radio

respirometry studies, it has been found that cells exposed to mercury dissimilated 100 % of [^{14}C]-methane and that provided reducing equivalents to fuel mercury (II) reduction (Boden and Murrel 2011). Thus, methanotrophs not only degrade the organic moiety but also aid in remediation of inorganic elements. There are reports that few methanotrophic bacteria produce extracellular polymers with the potential for industrial applications as well as for metal bioremediation. It is an important point to examine the broad-spectrum enzyme MMO of methanotrophs for its capability to detoxify/transform other toxic elements into a less toxic form as it depends upon the involvement of enzyme-mediated redox reactions (Valls and de Lorenzo 2002). Thus, the use of methanotrophic bacteria in the remediation of such toxic elements from contaminated sites could be an emerging tool in more sustainable way.

Environmental stresses and methanotrophic remediation

Adaptation mechanisms of methanotrophs at molecular level under various stresses viz. temperature, pH, salinity, sodicity, drought, and different types of chemicals are still not known (Jiang et al. 2010). Some other environmental factors well known to influence the population of methanotrophs are the ammonium and/or nitrite ions as they act as competitive substrates for MMO (Dunfield and Knowles 1995; Hanson and Hanson 1996). The exact mechanism of enzyme inhibition by nitrogen compounds and methane oxidation still needs to be understood. The main mechanism by which nitrogen inhibits methane oxidation is through ammonium, which competes with methane for binding site on MMO in methanotrophic bacteria. Although the affinity of MMO for methane is many folds higher than its affinity for ammonium, excessively high concentrations of ammonium is known to substantially inhibit methane oxidation (Bedard and Knowles 1989). However, recent studies with rice plants have revealed that nitrogen fertilization increases CH_4 oxidation in densely rooted soils because rhizosphere methanotrophs face intense plant and microbial competition for nitrogen (Macalady et al. 2002; Eller et al. 2005). The information about MMO-mediated methanotrophic remediation affected by nitrogenous compounds is almost lacking. Hence, impact of nitrogenous compounds on methanotrophic remediation needs to be investigated. However, modern research in 'omics' technologies (proteomic, metabolomic, genomic, soil metagenomic, and transcriptomic) may boost our understanding on the adaptation potential of methanotrophs in various habitats and their bioremediation potential in the presence of various stress conditions including the presence of organic and inorganic pollutants. To examine the impact of these environmental stresses on methanotrophs in relation to bioremediation potential, there is an urgent need for detailed studies to address these questions.

Genetic engineering in methanotrophs for enhanced bioremediation

With the aid of biotechnology and genetic engineering, bacteria have been exploited for in-situ bioremediation of a wide range of pollutants (Barac et al. 2004; Liu et al. 2011; Villacieros et al. 2005). Genetic engineering of indigenous bacteria, well adapted to local conditions, offers more efficient bioremediation of polluted sites (Singh et al. 2011). Genetic engineering in methanotrophs may provide opportunities to exploit the unusual reactivity and broad substrate profile of MMO for maximum benefit in the field of remediation technologies and to manipulate the tolerance, degradation potential of methanotrophs against various organic and inorganic pollutants through introduction of desired genes. Thus, the development and application of genetic engineering of the native methanotrophs will definitely offer more efficient and enhanced bioremediation of the pollutants viz. heavy metals, organics or co-contaminants, making the bioremediation more viable for environment remediation (Fig. 1). Some points have to be put in mind such as bio-safety assessment, risk mitigation, and factors of genetic pollution before using the genetically engineered bacteria at field level (Singh et al. 2011) including methanotrophs. However, the future application of genetically engineered methanotrophs for pollution remediation will not be free from the risks associated with their release in the environment. The future risk regarding use of such engineered bacteria is still unclear. The pathway of safe application of efficient bioremediation through genetically engineered methanotrophs: laboratory → bench scale → pilot scale → finally field scale testing → commercial field application, etc. is not a remote dream. There is need to develop novel methanotrophs through the biotechnological and genetic engineering approach to protect the environment.

Limitations to methanotrophic bioremediation

High cell density cultivation, number of culture conditions, and fermentation technologies for methanotrophs have been studied extensively. However, there are still several

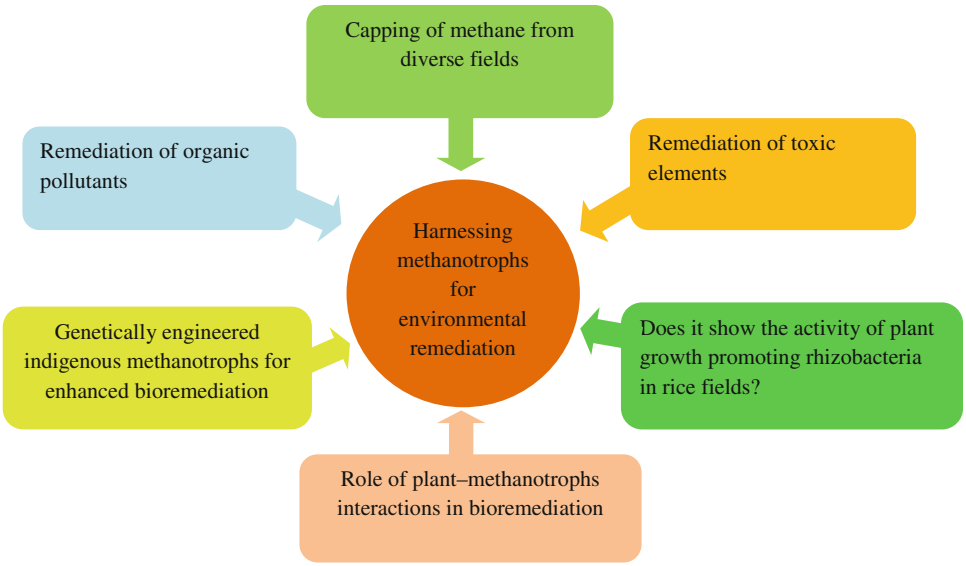

Fig. 1 A hypothetical model showing the application of methanotrophic bacteria for environmental remediation

limitations for methanotrophic study such as slow growing strains, low solubility of methane, and oxygen in aqueous phase (Jiang et al. 2010). It is general problem that toxic intermediates may be formed during the aerobic degradation of chlorinated compounds which cause damage to the bacterial cells. In this regard, utilizing a dichloromethane (DCM)-degrading microbe which has an important DNA-repairing mechanism in response to DNA damage caused by formation of toxic intermediate during the degradation of chlorinated compounds (Kayser and Vuilleumier 2001). This bacterium utilizes DCM as a sole source of carbon and energy. An aerobic bacterium was reported to degrade mono- to trichlorinated dioxins with a newly discovered enzyme, an angular dioxygenase known as carbazole 1, 9 a dioxygenase (Habe et al. 2001). For characterization of microbial populations, a new screening assay based on quinone profiling (a culture-independent lipid biomarker approach) has been developed for the analyses of in situ microbial populations in dioxin-polluted soils (Hiraishi et al. 2001). Knowledge of DNA repair mechanism (important physiological tool) in dioxin-degrading bacterial strain and bacterial screening assay in the dioxin-polluted soil may help us in our understanding and prediction of the methanotrophs-based degradation of chlorinated dioxins. Further more research is needed to eliminate these limitations for exploitation of these promising methanotrophs for human welfare.

Other research needs related to methanotrophs

It is well known that methanotrophs are ubiquitous in the environment and globally important in oxidizing the potent greenhouse gas methane. There is an urgent need to optimize the effect of agricultural practices on this microbe in different bio-geographical regions of the world. The population dynamic and diversity of the methanotrophs need to be studied with respect to edaphic and climatic conditions of environment. A few citations on these aspects have emerged recently (Vishwakarma et al. 2009; Zheng et al. 2008; Singh et al. 2010; Singh and Pandey 2013). Recently, plant–microbe interactions have been suggested as a promising technology to enhance phytoremediation (Rajkumar et al. 2012), which offers a future tool for potential application of methanotrophs for exploitation in other ecosystem services. Several plants involved in interactions with rhizosphere-associated microbes can be exploited to remediate toxic environments (Weyens et al. 2009). But plant–methanotrophs relations studied till date have mainly focused on rice fields and wetlands because of their importance as major areas of methane production (DeBont et al. 1978). But the role of plant–methanotrophs interactions in the field of remediation of toxic elements from contaminated sites is still little explored. Plants can also take benefit from these associations. Since methanotrophs can excrete or release phytohormones (cytokinins and auxins) after cell lysis and other bioactive compounds (Doronina et al. 2004), the plants can benefit from their association. Additionally, different aspects of methanotrophs were examined in the rice fields (Horz et al. 2001; Mohanty et al. 2007; Singh et al. 2010; Singh and Pandey 2013; Wu et al. 2009), but none of these were related to plant growth-promoting methanotrophs. So, there is still need to examine the plant growth-promoting activity of methanotrophs in agricultural fields. Similarly, methanotrophs

should be collected from different agro-climatic zones of the world for assessing their bioremediation potential. The collected methanotrophs should be cultured and used in different parts of the world for detailed morphological, genomic, and molecular characterizations. The fast-growing methanotrophs could be used for remediation of different types of pollutants. The database knowledge exchange program should be facilitated at the global level.

Remediation of soil pollutants by methanotrophs is emerging fast. Methanotrophic bacteria in forest soil exhibit the highest methane sink activity on a global scale (Dalal and Allen 2008), but their methane sink capacity decreases when natural land use pattern is altered (Dorr et al. 2010). Methane sink activity (kg ha^{-1} $year^{-1}$) by methanotrophs in different ecosystems is increased in order: sheep pasture (0.8) → shrub land (2.3) → disturbed forest (2.9) → pine forest (4.2) → tropical forest (4.6) → subtropical forest (5.5) → dry land paddy (5.8) (Singh 2011). Considering the immense potential of methane sink activity in tropical and subtropical forests, widespread salt-affected denuded wasteland (about 955×10^6 ha) in arid and semi-arid regions (Szabolcs 1994), there is a great potential of methane sink activity through afforestation program on these wasteland for better growth of these bacteria (Pandey et al. 2011).

Conclusion

Methanotrophs were discovered over a century ago; however, methanotrophs have not been explored well. The research related to bioremediation potential of methanotrophs is still in infancy stage. For better harnessing of methanotrophs in industrial application and bioremediation, a number of limitations need to be worked out such as lack of suitable cultivable methanotrophs and isolation techniques, competitive inhibition of methane mono-oxygenase by ammonium, low solubility of methane, production of toxic intermediates. With the combination of biotechnology and genetic engineering, methanotrophs can be exploited for in situ bioremediation of a wide range of inorganic and organic pollutants.

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