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Review

Biologically derived fertilizer: A multifaceted bio-tool in methane mitigation

Jay Shankar Singh [a,*], P.J. Strong [b]

[a] Department of Environmental Microbiology, BB Ambedkar (Central) University, Lucknow 226025, Uttar Pradesh, India
[b] Centre for Solid Waste Bioprocessing, School of Civil Engineering, School of Chemical Engineering, University of Queensland, St. Lucia, Queensland 4072, Australia

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ABSTRACT

Methane emissions are affected by agricultural practices. Agriculture has increased in scale and intensity because of greater food, feed and energy demands. The application of chemical fertilizers in agriculture, particularly in paddy fields, has contributed to increased atmospheric methane emissions. Using organic fertilizers may improve crop yields and the methane sink potential within agricultural systems, which may be further improved when combined with beneficial microbes (i.e. biofertilizers) that improve the activity of methane oxidizing bacteria such as methanotrophs. Biofertilizers may be an effective tool for agriculture that is environmentally beneficial compared to conventional inorganic fertilizers. This review highlights and discusses the interplay between ammonia and methane oxidizing bacteria, the potential interactions of microbial communities with microbially-enriched organic amendments and the possible role of these biofertilizers in augmenting the methane sink potential of soils. It is suggested that biofertilizer applications should not only be investigated in terms of sustainable agriculture productivity and environmental management, but also in terms of their effects on methanogen and methanotroph populations.

Contents

1. Introduction

Methane (CH_4) is a potent greenhouse gas that contributes towards global warming and is the second most important greenhouse gas, after carbon dioxide (IPCC, 2007; Zhou et al., 2013). Although less abundant than CO_2, CH_4 has approximately twenty times the impact (Kirschke et al., 2013). Anthropogenic activities such as the use of fossil fuels, agriculture, livestock farming, landfilling and biomass burning, account for 63% (566 Tg CH_4 yr^{-1}) of global CH_4 emissions (Bousquet et al., 2006; Kirschke et al., 2013). Therefore, increased anthropogenic activity affects the CH_4 flux and has a strong potential to impact climate change. Methanotrophs sequester atmospheric CH_4 in soils and are predicted to consume between 10 to 40 Tg CH_4 yr^{-1}, trapping more than 50% of the CH_4 produced in soils (IPCC, 2001; Reeburgh et al., 1993).

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[*] Corresponding author.
E-mail addresses: jayshankar_1@bbau.ac.in (J.S. Singh), pjstrong@gmail.com (P.J. Strong).

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Agricultural production is expected to double by 2050 to support a projected human population of 9 billion (Raja, 2013). Increased food requirements place greater pressure on agrarian practices, where crop yields and areas must increase to meet demand (Singh et al., 2011). Agricultural practices (including crop type, irrigation, organic amendments and fertilizer use) affect CH_4 production and consumption as these microbially-mediated processes are regulated by the prevailing soil physico-chemical properties of agrarian soils and sediments (Zheng et al., 2010). Nitrogen (N) fertilizers are widely recognized as one of the key factors affecting CH_4 oxidation in agricultural soils (Kravchenko et al., 2002; Seghers et al., 2003). While short-term use of N fertilizers may prevent enzymatic methane oxidation, long-term use affects soil microbial communities and can impact CH_4 production or oxidation (Bodelier and Laanbroek, 2004; Ho et al., 2014). A greater understanding of the effects of N fertilizers in the microflora responsible for the methane sink in soils is crucial to predict future trends in CH_4 emissions. Net CH_4 flux within the soils of agricultural lands may be governed by microbial interactions and subsequent changes to CH_4 producing and consuming microbial populations. Even in well-aerated agricultural soils, where low or negligible CH_4 uptake capacity was assumed, research indicates that CH_4 oxidation is more important than previously assumed (Ho et al., 2015). The rate of global methane emissions from agricultural activities will certainly increase further if production is increased and intensified and no mitigation strategies are adopted.

Agriculture is essential for food security and economic returns, but must be environmentally sustainable. Rice paddies are a significant source of CH_4, which is problematic as rice production alone is anticipated to increase from 600 million tonnes in 2000 to 930 million tonnes by 2030 (Kubo and Purevdorj, 2004). Although genetically modified rice strains that divert more carbon into the grains could potentially decrease CH_4 emissions (Bodelier, 2015), the foreseeable future of increased rice production will require more fertilizers to provide nutrients. It is important to increase agricultural production without detriment to environmental quality and even simple strategies can have great benefit. Adopting alternate wetting and drying cycles in rice production has delivered promising results, with up to 30% less CO_2-equivalent emissions (IRRI, 2015). Practices such as these may ameliorate problems of accelerated gas emissions resulting from N-fertilizer based agriculture. Another practice, where organic fertilizers are combined with microbial amendments (biofertilizers) is a promising alternative to inorganic fertilizers. Currently, there are few reports emphasising the prospective role of biofertilizers with regard to CH_4 mitigation, and this review article presents biofertilizers as a possible means to improve CH_4 mitigation in agricultural systems. It is pertinent as sustainable and efficient tools are required to mitigate CH_4 emissions *via* natural soil micro-flora (which include methanotrophs), while simultaneously improving soil quality and crop yields.

2. Overview of ammonia and methane-oxidizing bacteria

The interaction between CH_4 and nitrogen has been identified as one of the major gaps in carbon–nitrogen cycle interactions (Gardenas et al., 2011; Stein et al., 2012). So too is the interaction between the two pivotal functional groups: CH_4 oxidizers and ammonia oxidizers. The two groups are proposed to have a common evolutionary history as the enzyme systems of CH_4 and ammonium-oxidizers are similar and they occupy similar ecological niches (Holmes et al., 1995; Stein et al., 2012). To better understand the complex relationship between ammonia and methane-oxidizing bacteria, it is worth first describing these microbes and their key enzymes (Pandey et al., 2014).

Autotrophic nitrifiers are responsible for the oxidation of ammonia to nitrite, which is a key process in the global cycling of nitrogen (Singh and Kashyap, 2006). Ammonia monooxygenase (AMO) is responsible for the first step, where ammonia is oxidized to hydroxylamine (Hollocher et al., 1981). The majority of known isolates of ammonia oxidizers or nitrifiers are β-Proteobacteria and consists of the genera *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio* and *Nitrosolobus*. The second group is γ-*Proteobacteria* that consists of two species of the genus *Nitrosococcus* and is exclusively marine (Coci et al., 2008).

Methanotrophic bacteria can use methane as their sole carbon source and they serve as a global sink for methane. Methane monooxygenase is the key enzyme that is responsible for the first step, where CH_4 is oxidized to methanol. Methanotrophs are traditionally classified as Type I (γ-*Proteobacteria*) or Type II (α-*Proteobacteria*), primarily according to their use of the ribulose monophosphate pathway (Type I) or serine (Type II) pathways for formaldehyde assimilation). They were further subdivided into a Type X group, which used the ribulose monophosphate pathway of the Type I methanotrophs, but also had capabilities associated with Type IIs. Methanotrophs are now predominantly classified according to whether they are γ-proteobacteria or α-proteobacteria; Type X are regarded as a subdivision of Type 1 as they are γ-proteobacteria. Verrucomicrobium (*Methylacidiphilum* and *Methylacidimicrobium*), a recently-discovered phylum that consists of thermophiles, has also been added to the group of known methane oxidising bacteria (Stein et al., 2012; Kalyuzhnaya et al., 2015; Strong et al., 2015).

Methane monooxygenase and ammonia monooxygenase occur as particulate membrane-bound enzymes, although a soluble form of methane monooxygenase (sMMO) is synthesized in copper-deficient environments by some methanotrophs (Semrau et al., 2010). Both ammonia and methane monooxygenases have broad substrate ranges that include various hydrocarbons and halogenated hydrocarbons (Hyman et al., 1998; Vannelli et al., 1990). As both enzymes are capable of oxidising either ammonia or methane, methanotrophs and autotrophic nitrifiers are both participants in the global cycling of nitrogen and CH_4. Genes encoding particulate methane monooxygenase (pMMO) and ammonia monooxygenase share high sequence identity and, despite their different physiological roles, appear to be evolutionarily-related enzymes (Holmes et al., 1995). Methanotrophs and autotrophic nitrifiers share many similarities and have several common key enzymes.

In natural systems, the methanotrophs may play an important role in the N cycle, while the contribution of ammonia oxidizers to methanotrophy appears to be relatively minor. A similar trend was observed for rice crop soils. A study by Bodelier and Frenzel (1999) allowed for the discrimination methane and ammonia oxidation in using a competitive inhibitor (CH_3F) and by differential recovery after inhibition by C_2H_2. By applying both assays to model microcosms the authors demonstrated a major contribution of methanotrophs to nitrification in the rhizosphere, while the contribution of nitrifiers to CH_4 oxidation was insignificant. However, ammonia oxidizers may have a more important role in agricultural soils. Akiyama et al. (2014) measured N_2O and CH_4 fluxes after urea application in fields containing different soils and their results suggest that the ammonia oxidizers be more important to CH_4 oxidation than previously considered, regarding agricultural soils treated with N fertilizers. Despite their clear overlapping abilities, the complex relationship and interactions between methanotrophs and ammonia oxidizers in the laboratory are not well understood. Zheng et al. (2014) assessed competition between methane and ammonia oxidizing bacteria and observed transfer of C from CH_4 oxidation to other soil microbes, which was enhanced