Development of microbial inoculants for green house gases mitigation under irrigated rice ecosystem

Abstract

Our earth releases several types of green house gases into the atmosphere *viz.*, carbon dioxide, nitrous oxide, methane, chlorofluorocarbon etc. Rice is a prime food crop in the Asian countries which fulfil the food security. Methane is one among the greenhouse gases which usually emitted from low land rice field. Methanogen survive in the absence of oxygen and usually releases methane from irrigated rice ecosystem. Methanotroph are aerobic group of organism that require minor amount of oxygen to survive. Rice ecosystem is the ideal ecosystem for the survival of methanogens and methanotrophs. With this background methanotrophs were isolated from four different location of cauvery delta zone. Cultures were confirmed through methane monooxidase activity. Methanotroph isolates were mass multiplied and bioinoculants were developed.

Key words

Methanotrophs, MMOs activity, Compatibility assay, Mass multiplication, Bioinoculants

Abbreviations

MMOs : Methane Monooxidase activity

Introduction

"Methane is an important warming gas and its atmospheric concentration has been increasing for many decades" (Crutzen, 1995, Wassmann, 1993 and Khalil and Rasmussen, 1990). "Wetland rice fields appear to be one of the major sources of methane emissions to the atmosphere" (IPCC, 1992). "Methane emission from a rice field is the net eject of methane production (methanogenesis) and oxidation (methanotrophy). Methane genesis occurs by the anaerobic organism (Methanogen) and methane catching organism is available known as a Methanotrophs. Methanotrophic or methane-oxidizing bacteria (MOB) are an important group of bacteria that use methane as their sole source of carbon and electrons. There is an increasing interest in Methane Oxidation Bacteria (MOB) because of their importance in greenhouse gas consumption and their potential application in bioremedial degradation of industrial pollutants, e.g., trichloroethylene" (Hanson & Hanson, 1996). MOB needs both methane as electron donor, and oxygen as co-reactant in the oxygenase

reaction and as electron acceptor. In sediments, methane diffuses upwards from deeper sediment layers, and oxygen diffuses from the water column into the sediment. Both gases overlap at very low concentrations in the top few millimeters below the sediment surface where MOB can live in counter gradients of methane and oxygen. In this narrow zone, methanotrophic growth is limited by the diffusive transport of both substrates.

"Methanotrophs use methane monooxygenase (MMO) to catalyze the oxidation of their primary growth substrate methane to methanol. The enzyme can be found either in the cytoplasm in a soluble form (sMMO) or in the membrane bound particulate form (pMMO). The pMMO enzyme is found in all known methanotrophs, with the potential exception of an acidophilic methanotroph isolated" by Dedysh *et al*. (1998). "Of the organisms also capable of sMMO production, the majority have been found to be of the type II and type X methanotrophs affiliated with the subclass of *Proteobacteria"* (Hanson and Hanson, 1996). "Though rare, strains of type I methanotrophs (affiliated with -*Proteobacteria*) have also been found to produce sMMO" (Auman and Lidstrom, 2002.). "The *mmoX* gene encodes the conserved subunit of the hydroxylase component of the sMMO" (Shigematsu, 1999). Hence the methanotrophs were isolated specific to low land rice ecosystem and microbial consortium was developed for mitigation of green house gases

Methodology

Isolation of methanotrophs

"Soil samples were collected from ten different locations of low land paddy fields of Cauvery Delta Zone. Representative field soil samples were serially diluted to 10^{-3} in phosphate buffer and plated in the Nitrate Mineral Salt medium (Whittenbury, 1970). The plates were incubated in a fabricated McIntosh jar (Plate 1) upto 7-15 days connected to the bladder containing methane gas".(Bharathi et. Al., 2018)

Methane monooxygenase activity (MMO'S)

The microbial colonies, pale white with slimy nature containing petriplates were removed from the McIntosh jar and subjected to MMO's activity. The lids of petriplates were sprinkled with naphthalene powder and kept inversely such that the colonies at the bottom plate absorb the naphthalene gas from the lid for 15 minutes. After 15 minutes, the lid was once again placed inversely and the sprinkled naphthalene crystals removed. Then the colonies immersed with O – dianisidine solution and kept for 30 minutes. The pale white

colonies turn reddish brown or purple in colour that indicates positive reaction of MMOs activity as per the standard procedure given by Wackett and Gibson (1983).

Compatibility test of the isolates

"Methanotroph isolates were tested for compatibility by cross streak assay. Two different cultures were perpendicularly streaked in a single plate containing nitrate mineral salt medium. Totally 16 plates were used for four different combination of methanotroph culture" (Plate 2). (Bharathi et. Al., 2018)

Mass multiplication of methanotrophs

For mass multiplication of Methanotrophs, Nobel Agar Solid medium (Whittenbury *et al*., 1970), M_9 medium (Arie Geerlof –Helmholtzz Center Munich, 2010 or Sigma –Aldrich M6030) and basal medium (DSMZ –GMBH @ 2015) were used. The cultures were inoculated in the broth with and without carbon source and supplied with methane gas. Since the cultures were microaerophilic in nature (slow growth) the broths were kept upto 15 days (Plate 3).

Bioinoculants preparation

The best culture screened were mixed with each other after growing in the nitrate mineral salt broth with 10mM of phosphate buffer (pH 7) with 10% glycerol

Results

Isolation and confirmation of methanotroph

Methanotrophs were isolated using nitrate mineral salt medium and confirmed through MMO's activity test. Round shaped, brown colour colonies were observed after 15 days of inoculation. Among the ten isolates, isolates from Aduthurai, Needamangalam, Keelamaruthuvakudi and Vadagarai showed positive result towards MMOs activity. The results revealed that all the four cultures (Aduthurai, Vadakarai, Keelamaruthuvakudi and Needamangalam) were compatible with each other.

Mass multiplication and screening of the isolates

Different growth media with supplements as shown below were tried to find out the one which supports the maximum growth of methanotrophs. Among the different growth medium used nitrate mineral salt medium + glucose, basal broth+ Methanol+ Glucose and M_9

liquid medium gave maximum growth than the other medium. Among the cultures, isolates from Keelamaruthuvakudi and Aduthurai exhibited better performance for growth and MMOs activity. Hence, it was screened for bioinoculants preparation.

List 1 : Mass multiplication and screening of the isolates

Medium	**Maximum growth**
M_2 medium	No growth
M_2 medium+ Glucose	Slow growth
Nitrate mineral salt medium	Moderate growth
Nitrate mineral salt medium + Glucose	Good growth
Basal broth+ Methanol+ Glucose	Good growth
M_9 –liquid medium	Good growth

Bioinoculant Preparation

Among the isolates, cultures from Keelamaruthuvakudi and Aduthurai showed better performance than Needamangalam and Vadakarai. Hence these cultures were separately mass multiplied in the Nitrate Mineral Salt medium supplemented with glucose and finally mixed. 10mM of phosphate buffer with 10% glycerol was added as a cell protectants

Discussion

A measure to control CH_4 emission from paddy fields mitigation options have been reported (Lu *et al*., 2000), which included 1. Organic matter management 2.water management 3.Selection of rice cultivar and 4. Chemical fertilizers. “Improving organic matter management by promoting aerobic degradation through composting or incorporating into soil during off-season is another promising technique” (Yagi *et al*., 1997). Espiritu *et al*., (1997) mentioned “the population fluctuations of methanotrophs in paddy soil and rice rhizosphere are likely to be closely affected by the supply of O_2 namely reduced or oxidative conditions at each stage of rice cultivation”.

Methanotrophic bacteria in paddy fields are beneficial in two ways 1. CH_4 oxidation to CO_2 in paddy fields is an efficient means to mitigate global warming and 2) group II methanotrophs which are capable of fixing of exospores or cyst as dormant bodies, seems to be dominant in rice soils under low $-O_2$ and high CH_4 conditions (**Dianou, D. and K Adaci. 1999**). They may also support potential N fertility in paddy fields.

Whittenbury and Dalton (1981) and Murrell (1992) suggested that "of the 11 methanotrophs tested so far fix nitrogen while some species of group I methanotrophs are also able to fix nitrogen" (Hanson and Wattenberg, 1991). Rice root systems affect CH_4 production and oxidation in the rice rhizosphere and their influence varies with different rice cultivars. Here we assume that in seeking measures to control CH_4 emissions from paddy fields in relation to rice cultivars, the root oxidative activity or niche for methanotrophic bacteria in the root areas is important (Dianou *et al.*, 1997).

This was supported by the finding of Conrad and Rothfuss (1991) who reported "methane oxidation an important process which reduced the CH_4 flux from wetland rice fields. Methanotrophs typically oxidize 70-90% of the CH_4 diffusing upward through the oxic soil surface layer in most soils". Hence the methaotrophic bioinoculants were prepared with cell protectants to mitigate green house gases in low land rice field. This result supported the findings of Lorda and Balatti (1996), who reported "glycerol, is capable of enhancing cell tolerance". "Glycerol has a high water binding capacity and may protect cells from the effect of desiccation by slowing the rate of drying. Glycerol and PVP hold large amount of water and protects cells from effect of desiccation by slowing the rate of drying" (Tamil Vendan and Thangaraju, 2006).

Conclusion

Global warming is a great challengeable task for the human being to avoid environmental pollution and climate change. Irrigated rice ecosystem and cow rumen are the major source of methane gas emission due to the anaerobic environment. Exploitation of methanotroph and alternating the ecosystem by creating aerobic environment can be possibly applied to minimize the release of methane gas via anaerobic organism.

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Plate 1. Fabricated McIntosh jar

Plate 2. Compatibility test of the methanotroph isolates

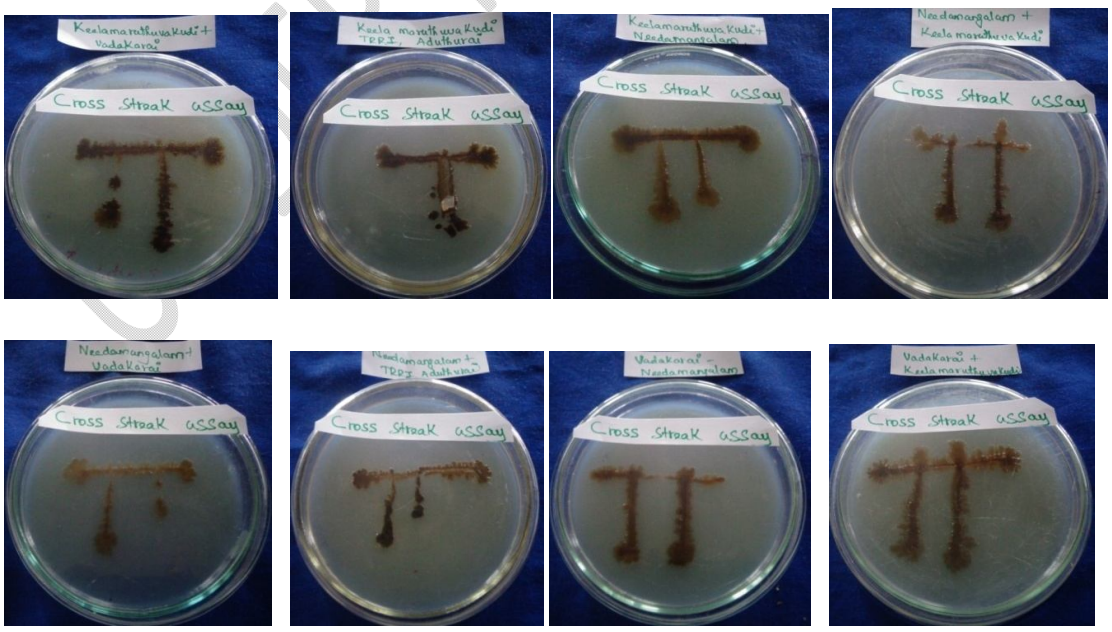

Plate 3. Mass multiplication of the methanotroph isolates

NSM broth Basal broth M_9 broth