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DYNAMICS OF METHANOGENS IN REDOXIMORPHIC SOILS OF NORTH WESTERN HIMALAYA

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ABSTRACT

Methanogenesis in redoximorphic soils involving methane efflux from soil to atmosphere is the result of microbial mediated process. Maiden attempt was made to understand dynamics of methanogens in the redoximorphic soils of Kashmir. The population of methanogenic bacteria was low during the early growing stages of rice that is at active tillering stage then attenuated gradually at pre heading stage in the range of $1.9x10^5$to$1.2x10^5$ g-1 dry soil. A variable methane flux was observed in the different treatments involving organics and inorganics alone or in proper blend. Methanogens preferd substrate carbon from aquatic weeds from the Dal Lake exhibiting highest methane emission values during rice growing season. Paddy straw blended with cellulolytes displayed methane flux up to 0.60 kg ha^{-1} day^{-1} displaying some regulation in methanogenesis by coexistence of methanogens in the niche of cellulolytes. The positive tubes dominated by rod-shaped cells about 1.4 mm long and about 0.4 mm in diameter showed the strong blue fluorescence typical of methanogens due to F420.

Key words: Methanogens, redoximorphic soils, Kashmir, aquatic weeds

INTRODUCTION

Methanogenesis in submerged rice soils is the result of complex interactions between rice plants and soil microorganisms under anoxic and reduced conditions prevalent in such soil besides rice rhizosphere is nourished by organic carbons which derive from root exudates and decaying roots during the growing season (Dannenberg and Conrad, 1999; Lehmann Richter *et al.*, 1999; Bhat and Beri,1996). Added organic matters in soil is hydrolyzed to fermentable sugars by variety of microbial enzymes (Kulkami *et al.*, 2007). A consortium of soil microbes consisting of fermentative, acetogenic and acetate-utilizing bacteria, involved in the degradation of organic matter in flooded rice fields, lead to the production of CO_2, H_2 and acetate, which produces methane by methanogens. Approximately 67 and 33% of the methanogenesis is from precursors viz acetate and H_2/CO_2, respective (Neue *et al.*, 1996; Lehmann *et al.*, 1999; Yao and Conrad, 1999; Martin *et al.*, 2005). Submerged rice soil and sediment are reported as the important niche of greenhouse gas produced by methanogenic Archaea (Conrad *et al.*, 2006). Wetland rice cultivation had been a normal practice in Kashmir division covering 1.60 lakhs hectare (approx) that is likely to increase owing to the growing demand for food by

increasing population; which is most likely to meet by the existing cultivated wetland rice area through intensifying rice production in all rice ecologies. No endeavor has been made to study the methanogens in wetland rice ecologies of Kashmir so far. Speculating uncertainties in quantification of methane, it has necessitated generating maiden data from this part of India for computing total methane efflux (Bhat and Beri, 1996), for the rice ecologies of India.

MATERIAL AND METHODS

Methane emission was investigated in the field experiment conducted at the Research farm of S K University of Agricultural Sciences and Technology of Kashmir, Shalimar campus. The physico-chemical properties of soil was pH – 6.9, EC (dsm^{-1})-0.05, O C(%) – 0.96, Available N (Kg ha-1)-.210, Texture Silty Clay Loam. Soil temperature varied between 10 to 28.5°C during growth period. The treatments were as: Control – T_0, Ammonium Sulphate (100%N)-T_1, Ammonium Chloride (100%N)-T_2, Ammonium Nitrate (100%N)-T_3, Paddy straw + Urea (100%N)-T_4, PaddyStraw + Cellulolytes-T_5, Aquatic weeds-T_6.

Each treatment had three replications. A plot size 3 × 2.5m. Treatments were in randomized block design. Application of FYM, paddy straw, aquatic weeds and different blends as per treatment were incorporated one week before transplantation. Whereas treatments T_2 to T_4 were maintained at I DAT. Rice nursery (var. SKAU-23) of 25 days old was transplanted in above treatments on 21-06-2003.

Bottomless plexi chambers (100× 50 × 30 cms) were fitted on base. Each gas sampling chamber consisted of a permanently installed base unit (open bottom) and removable top. Flood water was used to seal the top to the base unit during gas collection. A rubber septum was fitted into chamber through which a gas collection was made. A water level was never allowed to recede below the base unit. Each chamber was fitted with thermometer to record ambient temp. Soil temp. and redox measurements were done at the time of a gas collection. Each chamber contained 11 rice seedlings at 4 to 5 leaf stage. A gas collection was removed through rubber septum with gas tight syringes with stainless steel hypodermic needles. Collected gas samples were immediately transferred to evacuated gas vacuutainers upon gas collection. Top was removed for equilibration every time after collection.

A Shimadzu 14 BPSTF gas chromatograph fitted with flame ionization detector was used to determine methane concentration of the headspace gas samples. A 0.1 ml unknown gas samples was injected into the stainless steel column Porpaq. Q .The temperature for oven, injector and detector was maintained as 80, 100 and 120 C^{05}. All the gas samples were analyzed for methane within four hours of collection. Standard gas methane was used for comparison. Methane gas appeared after retention time of 1.1 minute. The chromatograms were processed by omega software. Methane flux was estimated using closed chamber equation.

$F = (V/A) (\text{“}C/\text{“}t)$

Where F = methane gas flux, Vis volume of headspace chamber. A is soil surface under chamber. "C/"t is change in concentration per unit of time.

Sampling method For quantification of methanogens in the different treatments by most probable number (MPN) technique, soil sample (0-15 cm) was taken under the chamber and transported to the laboratory under anoxic conditions and were incubated under N_2 gas and the sterile basal medium (Zhang and Noike, 1991). Anaerobiosis was created by flushing vials with O_2-free N_2 gas. The vials were closed with rubber stopper and aluminum cap, incubated at 28° C for 4 weeks. MPN population of methanogens was determined on the basis of positive and negative tubes and positive tubes were used for calculations by refering standardized MPN table to determine methanogens per unit weight of rhizospheric soil sample. The methanogenic activity was calculated by linear regression of the increase in methane production in different treatments.

For microscopic observiation of the methanogens ,5 ml of 10^{-5} or 10^{-6} dilution was added into 4.5 ml of melt agar complex medium for methanogens and tubes were flushed with H_2 and injected 3 ml CO_2, incubated at 35°C for 15 days The colonies on agar medium were observed by fluorescent microscope . Colonies with presence of blue-green fluorescence and emission of methane as monitored by gas chromatograph. The colonies with a presence blue-green fluorescence and methane, was picked up into liquid medium for methanogenic bacteria by Hungate technique and incubated at 35°C for 10-15 days. The purity of liquid culture was checked by microscope and aerobic cultivation.

RESULTS AND DISCUSSION

Serial liquid dilution cultures were used for an estimation of the population of methanogenic microorganisms that could be cultured by measurement of the production of methane and calculation of the number of viable organisms by a statistical treatment. The Population of methanogens in paddy rice soil with application of different treatments during various stages of rice growth is shown in fig 1. The population of methanogenic bacteria was low during the early growing stages of rice that is $1.5x10^4$,$1.6x\ 10^4$,$1.4x10^3$,$1.5x10^4$ g-1 dry soil at active tillering stage in To,T_1,T_2,T_3,T_4 and T_5 and which increased gradually at pre heading stage to $1.9x10^5$,$2.2x10^6$,$1.8x10^5$,$1.3x10^5$,$1.2x10^5$ g-1 dry soil, At physiological maturity population methanogens dwindled in the range $1.9x10^3$ to $1.4x10^3$ g-1 dry soil. This was consistent with that the fluxes of methane emission were the highest. A rate of methane flux was observed varying between 0.10 to 0.81 kg ha^{-1} day^{-1} in T_0 during growth period and decline in methane emission was set in post 70DAT.

The positive tubes were dominated by rod-shaped cells about 1.4 mm long and about 0.4 mm in diameter which showed the strong blue fluorescence typical of methanogens due to F420 Methanogenic population observed in the soils were significantly higher in the Treatments where urea was added led to attenuated methane flux in comparison to control that

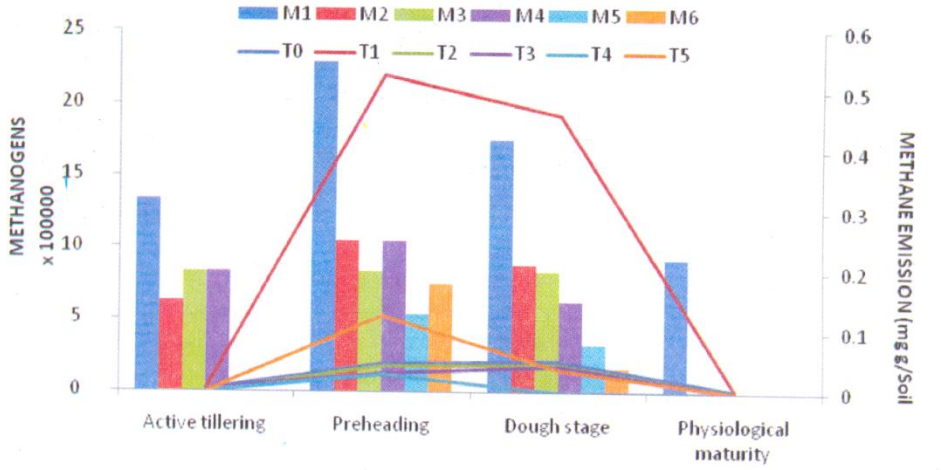

Fig. 1. Methane efflux and methanogens at different growth stages of rice

varied between 0.20 to 1.20 kg ha^{-1} day^{-1}. A methane flux was observed somewhat depressed in T_2, T_3, and T_4, treatments and was variable between 0 to 0.29, 0 to 0.28, and 0 to 0.30 Kg ha^{-1} day^{-1} in these treatments respectively through 90 days period of paddy cultivation that was consistent with a population dynamics. Paddy straw blended with cellulolytes displayed methane flux in the range of 0.00 to 0.60 kg ha^{-1} day^{-1} displaying some regulation in methanogenesis by coexistence of methanogens in the niche of cellulolytes. Aquatic weeds alone and blended with FYM a local practice with farmers ,emitted methane in the range of 0 to 0.90 and 0.45 to 0.95 kg ha^{-1} day^{-1} respectively indicating that aquatic weeds have stimulating effect on methanogenesis that is indicated by increased population of methanogens ($2.8x10^{11}$) using this substrate. Although the magnitude of the flux was different among the plots, but the pattern of peak emission was a little different in case of T_0 and T_1 treatment, first peak was observed on 20 DAT (first weak of July) whereas in case of T_2, T_3, T_4 treatments peaks were observed at 15 DAT (first weak of April) Two other small peaks were observed at 60 & 70 DAT. Further more, after continuous irrigation was interrupted and paddy field was intermittently drained at the end of August, the flux rapidly dropped. This trend is significantly correlated (r = 0.531**, n 24) with methanogens population.

There was a peak of methane production from rhizospheric soil at reproductive phase and another small peak at vegetative phase of day rice production in submerged rice soil of Kashmir. Similar result The high production of methane of rhizospheric soil at vegetative phase and reproductive phase may account for intensive metabolic activity, the variation of methanogens and associated microbial consortium and

amount of organic materials as substrates for methane formation. Therefore microbial consortium in rhizospheric soil and their activities related to available substrates were investigated and compared between vegetative phase and reproductive phase at day.

The rice plant also provides methanogenic substrates through root exudates into rhizosphere as a major source for methanogenic and associated microbial consortium in production and emission of methane from rice soils. Kaku *et al.* (2000) indicated that several substrates such as saccharides, amino acids and organic acid were provided from rice rhizosphere during the growing period of rice.

Methane oxidation mediated mainly by methanotrophic bacteria, which is strongly inhibited by ammonium accessible for nitrification as has been observed in the present studies (Hütsch, 2001). Indeed, in many studies NH4 was identified as a strong inhibitor for CH4 oxidation (Bronson and Mosier, 1994). Root derived organic C can contribute to various C pools and become an origin of CH4 emitted from flooded soils (Lu *et al.*, 2000). In the treatment where ammonium sulphate is added, methanogen population is low, because sulfate-reducing and methane producing organisms compete for the same substrates (H_2/CO_2, acetate-competitive substrates) but the sulfate reducers have the competitive advantage: they have stronger affinity to the competitive substrates and can use them to provide more energy than the methane producers (Schönheit *et al.*, 1982).

Change of substrates in at vegetative and reproductive phase of rice soils: The rice plant also provides methanogenic substrates through root exudates into rhizosphere as a major source for methanogenic and associated microbial consortium in production and emission of methane from rice soils. Kaku *et al.* (2000) indicated that several substrates such as saccharides, amino acids and organic acid were provided from rice rhizosphere during the growing period of rice. During rice early growing seasons, rice cultivars have the different nutrient requirement, which in turn, affect the inherent substrates and the fertilizers as exogenous substrates for CH4 production rate of planted soil (Holzapfel-Pschorn *et al.*, 1986). During rice late tillering stage, the rice exudates dominate CH_4 production rates of planted soil (Watanabe *et al.*, 1997). Methanotrophic microbial community apparently affected methane fluxes and hence that microbial diversity has to be taken into account in the global biodiversity debate.

Insreased methanogens in treatments where organic matter has been added is due to that the growth of soil microorganism is strongly controlled by the content of available organic carbon in soil. Long-term application of organic matter and inorganic fertilizer increased the numbers of microbes by the plate count method in soil (Kanazawa *et al.*, 1981; Kanazawa *et al.* 1988). Methanogenic substrates are produced by anaerobic decomposition of organic matter in paddy soil (Inubushi *et al.* 1984).

CONCLUSION

The culturable methanogenic group enumerated through MPN technique of microorganisms probably represents the

active microbial communities. However, population density of the studied microbial groups and their relationships with soil properties provides valuable insights to understand and interpret the variations in production and consumption of CH_4. Recent advancement in techniques may better link the microbial populations and their potential activities, such as the molecular approach based on the target genes that code the enzymes responsible for CH_4 metabolisms (Henckel *et al.* 1999; Braker *et al.* 2000) in rice growing soils of Kashmir. The continuous effort in this direction will help to develop a process-based model in the future to understand the mechanisms of CH_4 in rice soils of Kashmir in order to minimize their emissions to the atmosphere.

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