Table 2. MIC of papulacandin preparation against *C. albicans* by broth microdilution method using Sabouraud broth

Incubation time (days)	MIC (μg/ml)	
	Sabouraud broth	Sabouraud broth + sorbitol
2	1	4
5	2	> 64

allowed to remain on the shaker for two hours. This whole broth (W.B.) in methanol was centrifuged and 50 μl of supernatant, which was a representative of metabolites present in both culture filtrate and mycelium, was added per well in the two bioassay plates. The plates were left open in the laminar flowhood to allow evaporation of methanol from the test sample.

One of the fungal cultures, No. Y 97 07633 screened in this assay showed a reduced zone in the presence of sorbitol (Table 1), indicating the presence of a CW-active antifungal agent. The CW-active components from the fungal culture were identified by the process of dereplication[8] to be a mixture of papulacandins B and C. The papulacandin complex was enriched from the mycelium of the producing fungal strain by the use of chromatographic techniques such as Diaion HP-20, Silica gel and Sephadex LH-20. MIC of the papulacandin preparation was determined by broth microdilution method in Sabouraud broth and was found to be higher in the presence of sorbitol (Table 2), thus confirming the results of the crude extract obtained by the agar plate assay described earlier. Frost *et al.*[5] have also reported MIC values of 1.0 μg/ml for papulacandin B by SPAM.

Hence, the agar plate assay is rapid, convenient and sturdy and suited for high-throughput screening of natural products or synthetic chemical compounds, since results are available in 18 h and samples containing solvents like methanol can be tested. It can also be used for monitoring CW-active metabolites during its purification from microbial sources. Natural product or synthetic chemical samples can be tested directly by this agar plate method without knowing their MIC values. The assay replaces YNB with Sabouraud agar, which is economical and less cumbersome. The assay is neither sensitive to the density of the inoculum nor does it need the test sample to be sterile when extracted in methanol.

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Spatial variation in the capacity of soil for CH_4 uptake and population size of methane oxidizing bacteria in dryland rice agriculture

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The pattern of methane (CH_4) oxidation and population size of methane oxidizing bacteria (MOB) were studied in three different soils (rhizosphere, bulk and bare) of a dryland rice (*Oryza sativa* L. cultivar Narendra-118) field. The rhizosphere soil exhibited the strongest CH_4 oxidation activity and bare soil the weakest. MOB population size was significantly higher in the rhizosphere (671.0×10^5 cells g^{-1} soil) than in the bulk (569.0×10^5 cells g^{-1} soil) or the bare soil (49.2×10^5 cells g^{-1} soil), and NH_4^+-N concentration was highest in the bare soil (6.74 μg g^{-1} soil) followed by the bulk (5.58 μg g^{-1} soil) and rhizosphere soil (4.02 μg g^{-1} soil). Half saturation constant (K_m) and maximum oxidation rate (V_{max}) decreased significantly from the rhizosphere to bulk to bare soil and ranged from 84.01 to 5.81 μg g^{-1} dry soil and 0.62 to 0.05 μg h^{-1} g^{-1} dry soil, respectively. The rice rhizosphere not only supports a larger population of MOB but also contributes substantially to the capacity of soil for CH_4 uptake, leading to a predictable spatial pattern in CH_4 sink strength within the dryland rice ecosystem.

METHANE (CH_4) is an important greenhouse gas and plays a significant role in atmospheric chemistry[1,2]. Rice cultivation, a major anthropogenic source of methane, contributes about 20–150 Tg CH_4 yr^{-1} to the global budget[3]. Uptake by soil as a result of consumption by methane oxidizing bacteria (MOB) is a substantial sink for CH_4 (ref. 4). Even in flooded rice soil about 80 per cent of the CH_4 produced is oxidized *in situ*[5], with rhizosphere as the key site[6,7]. Dryland rice cultivation has been earlier demonstrated as a net sink for atmospheric CH_4, with rice

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plant as a component of this sink[8,9]. The rhizosphere in dryland rice fields has been recognized as an important microsite for colonization and multiplication of MOB[10]. It remained to be seen whether or not a larger MOB population confers on rhizosphere soil a greater capability of CH_4 uptake. In the present study we assess the capacity of CH_4 uptake and determine the kinetics (the half saturation constant, K_m, and the maximum oxidation rate, V_{max}) of CH_4 oxidation in the rhizosphere, bulk and bare soils in dryland rice agriculture. We also determine the population size of MOB and concentration of NH_4^+-N in these soils.

Investigations were carried out in the dryland rice field of the Botanical Garden, Banaras Hindu University, Varanasi, India (25°18′N lat. and 83°3′E long., 129 m above msl). The region has a seasonally dry tropical climate with a typical monsoonal character with the year divisible into a cold winter (November–February), a hot summer (April–June) and a warm rainy season (July–September). The annual rainfall averages 110 cm, of which 85% falls during the rainy season from the southwest monsoon[11]. The soil is a well drained inceptisol, pale brown, silty loam (sand 32%, silt 65% and clay 3%) with pH 7.8, organic C 0.59–0.70% and total N 0.06–0.09%.

The experiment was laid down in a completely randomized block design with 6 plots, each 15 m^2 in size. At the time of ploughing basal treatment of KCl + P_2O_5 + farmyard manure was applied at a rate of 60 : 60 : 1000 kg ha^{-1} to all plots. Three plots were vegetated by a dryland rice (*Oryza sativa* L.) variety, Narendra-118 and three were kept bare. These plots have been maintained with same treatments for the past three years (i.e. bare plots remained bare and vegetated plots were sown with Narendra-118 variety of rice each year). Seeds were sown by dibbling on 12 July 1999 at a spacing of 15 cm (hill to hill) by 20 cm (row to row) in the plots designated as vegetated plots. No irrigation was provided during the experimental period and the sole source of water was rainfall. The soil was sampled separately from each plot. The rhizospheric soil was collected by gently uprooting the plants and tapping the roots on a plastic sheet[12]. Samples of both bulk (between the plant rows) and bare (bare plots) soils were collected (0–10 cm depth) using a soil corer with 5 cm diameter. One part of each sample was weighed and oven-dried at 105°C to determine the moisture content. The other part, stored at 4°C, was used for chemical and microbial analysis within 2 days after sampling. The soil sampling was carried out at 40 days after sowing.

Ammonium-N was extracted by 2 M KCl and analysed by the phenate method[13]. The concentration of NH_4^+-N was significantly lower in the rhizosphere soil when compared to the bulk and the bare soils (Table 1). This may be due to uptake by rice and uptake and oxidation by microorganisms such as ammonium and methane oxidizers[14].

The number of MOB was enumerated by the MPN (Most Probable Number) technique[15]. In brief, fresh soil (5 g) was suspended in 15 ml of a modified ammonium mineral salt medium[16] with a trace element solution[17] and shaken for 12 h at 4°C in the dark. This suspension served as the inoculum. Instead of microtiter plates, culture tubes were used and dilution was carried out from 10^{-1} to 10^{-9} as described by Espiritu, Adachi and Senboku[18]. There were 6 replications for each dilution. After inoculation under aseptic conditions, the tops of the tubes were closed with sterilized cotton plugs. The tubes were incubated under 20% CH_4 in air at 25°C in the dark in an atmosbag for three weeks. For control, culture tubes were prepared without soil inoculum[18]. Further details are given elsewhere[10]. The population size of MOB was highest in the rhizosphere and least in the bare soil (Table 1), confirming our earlier study that rhizosphere provides the most favourable site for the occurrence and multiplication of MOB[10]. The soil of dryland rice fields gets periodically saturated due to heavy rainfall when CH_4 emission instead of net consumption occurs[9]. Under such a condition, the O_2-supplying potential of rice roots is a major factor for the multiplication, growth and sustenance of MOB. The supply of both CH_4 and O_2 would thus favour the MOB population[19] to develop in the rhizosphere than in the bulk or bare soils. The rhizosphere soil was also characterized by low NH_4^+-N concentration. We have earlier reported a negative relationship between MOB population size and NH_4^+-N concentration[10].

The capacity of the soil for CH_4 uptake was assessed in a CH_4-enriched environment[20–22]. Field-moist soil samples after an equilibration period of 24 h at 25°C were transferred to Erlenmeyer flasks (10 g to each flask, volume of the flask being approximately 135 ml). The flasks were sealed with a rubber stopper fitted with a silicon septum which allowed the injection of CH_4 and the sampling of headspace gas[21]. Control bottles without soil were incubated under the same condition. Soil samples with or without autoclaving were used to confirm whether or not the CH_4 oxidation was microbially mediated[22]. For autoclaving soil samples were wrapped loosely in aluminium foil and autoclaved for 1 h at 120°C. There was no visible change in the physical structure of the soil. Initial

Table 1. Comparison of kinetic parameters (K_m and V_{max}), MOB population size and NH_4^+-N concentration in soils of dry land rice field

Parameters	Rhizosphere soil	Bulk soil	Bare soil
K_m (μg g^{-1} dry soil)*	84.01	10.21	5.81
V_{max} (μg h^{-1} g^{-1} dry soil)	0.62	0.09	0.05
MOB population (× 10^5 cells g^{-1} dry soil)	670.96	568.93	49.21
NH_4^+-N (μg g^{-1} dry soil)	4.02	5.58	6.74

*These values convert to 345.27, 41.62, 23.46 μM, respectively, for rhizosphere, bulk and bare soils, where M indicates mol lit^{-1}; Values in each row are significantly different from one another at $P < 0.05$.

concentration in the headspace was adjusted to about 1150 ppmv by injecting the appropriate volume of pure CH_4 (99.99%). The actual initial concentration of CH_4 was determined by analysis. The flasks were incubated at 30°C in the dark for 10 days[21]. Headspace samples (0.1 ml) were taken with a gas-tight glass syringe after 0, 40, 90, 140, 190 and 260 h of incubation. These samples were analysed for CH_4 by a gas chromatograph (Nucon 5765); a flame ionization detector was used with a Porapak N column and helium as the carrier gas (flow rate 40 ml min^{-1}). Column, injector and detector temperatures were 40, 110, and 180°C, respectively. The experiments were done in triplicate.

There was no significant change in the CH_4 concentration in the headspace of the blank flasks (i.e. without soil) or those containing autoclaved soil, but the same declined substantially in flasks containing non-autoclaved soil samples (Figure 1). This confirmed the involvement of a biological process (i.e. consumption by CH_4 oxidizing microorganisms). Maximum CH_4 uptake was found in the rhizosphere soil (Figure 1), which had the largest population size of MOB.

The kinetics of CH_4 oxidation were obtained from data on the headspace concentration of CH_4 over time. Third-order polynomials fitted the data adequately ($r^2 = 0.989$–0.999). The rate of CH_4 oxidation (– dCH_4 conc/dtime) at any desired concentration was obtained by differentiation of the polynomial equations. The Michaelis–Menten kinetics was applied, and the kinetic parameters were determined by Lineweaver–Burk plots[23] for all the soil samples (Figure 2). Linearity of the plots ($1/CH_4$ oxidation rate vs $1/CH_4$ conc.) confirmed that the oxidation of CH_4 followed the Michaelis–Menten kinetics. From the slope and intercept values of the best-fit lines, V_{max} and K_m (apparent) were calculated using the equations: V_{max} = 1/intercept and K_m = slope/intercept. The values thus obtained are reported in Table 1. Higher K_m and V_{max} values were found for the rhizosphere soil when compared to the bulk and bare soils. V_{max} values indicated decreasing activity of methanotrophs from the rhizosphere to the bare soil. Wang, Zeng and Patrick[24] found a decreasing trend of K_m (165 to 4.1 µg g^{-1}) and V_{max} (12.5 to 1.2 µg h^{-1} g^{-1}) along the soil depth with decreasing CH_4 uptake. V_{max} values for cultivated and non-cultivated cambisol[25] ranged between 0.01 and 0.06 µg h^{-1} g^{-1}. The K_m value for CH_4 measured with purified methane monooxygenase (MMO) was 3 µM (ref. 26), and those for pure cultures of methanotrophic bacteria ranged between 1 and 66 µM (ref. 27). The differences in K_m and V_{max} among the three soils in this study could be due to differential species composition of the CH_4 consuming community and/or due to preconditioning of MOB under different soil microenvironments. For example, concentration of NH_4^+-N was significantly lower in the rhizosphere soil followed by the bulk and bare soils (Table 1). Ammonium has both a direct and indirect effect on CH_4 oxidation[28].

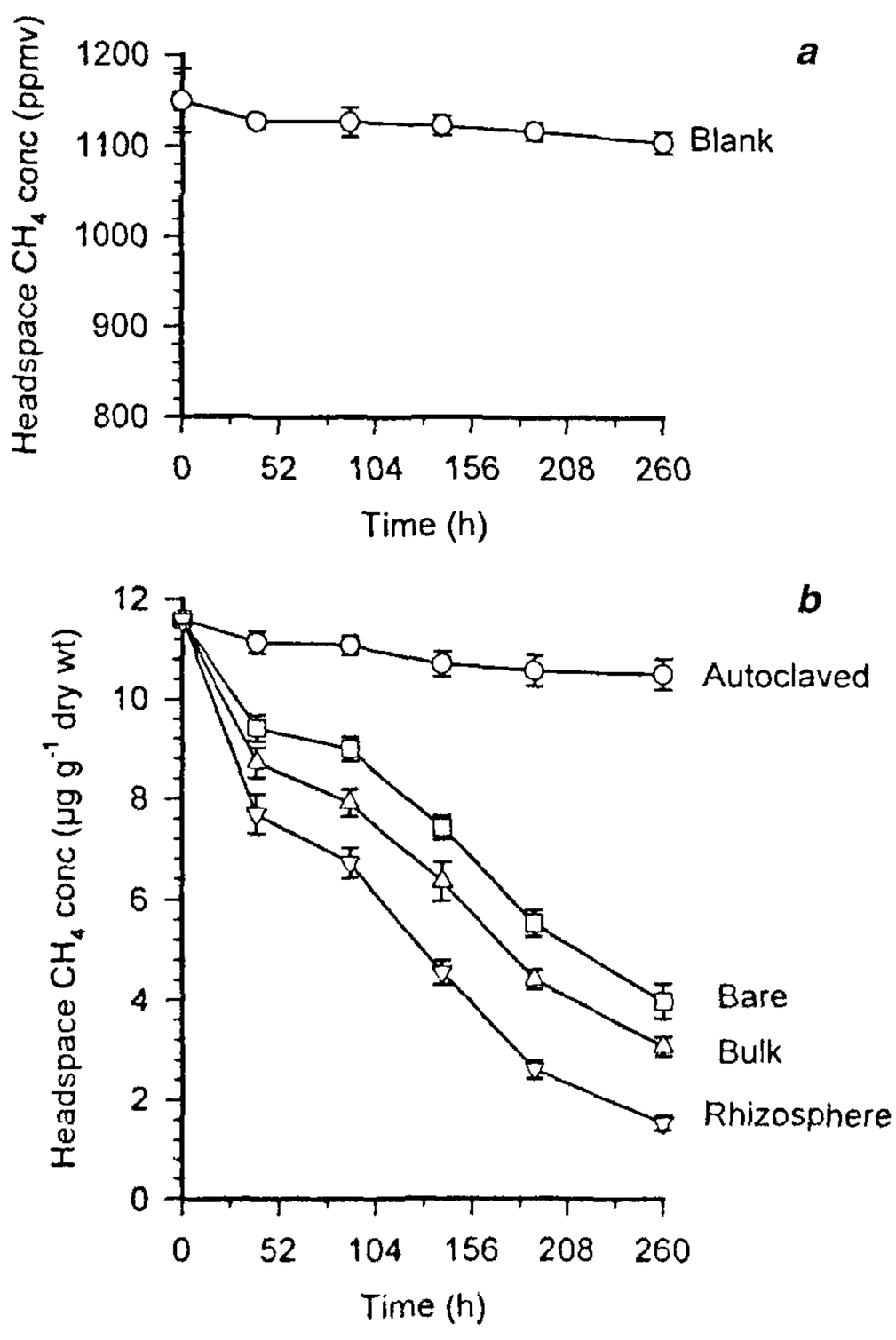

Figure 1. Time series of CH_4 concentration in the headspace of flasks, ***a***, without soil; and ***b***, with autoclaved rhizosphere soil and non-autoclaved samples of rhizosphere, bulk and bare soils. Values in ***b*** are presented on per g dry weight basis to eliminate variations due to moisture content of the samples. Vertical bars are ± 1 SE.

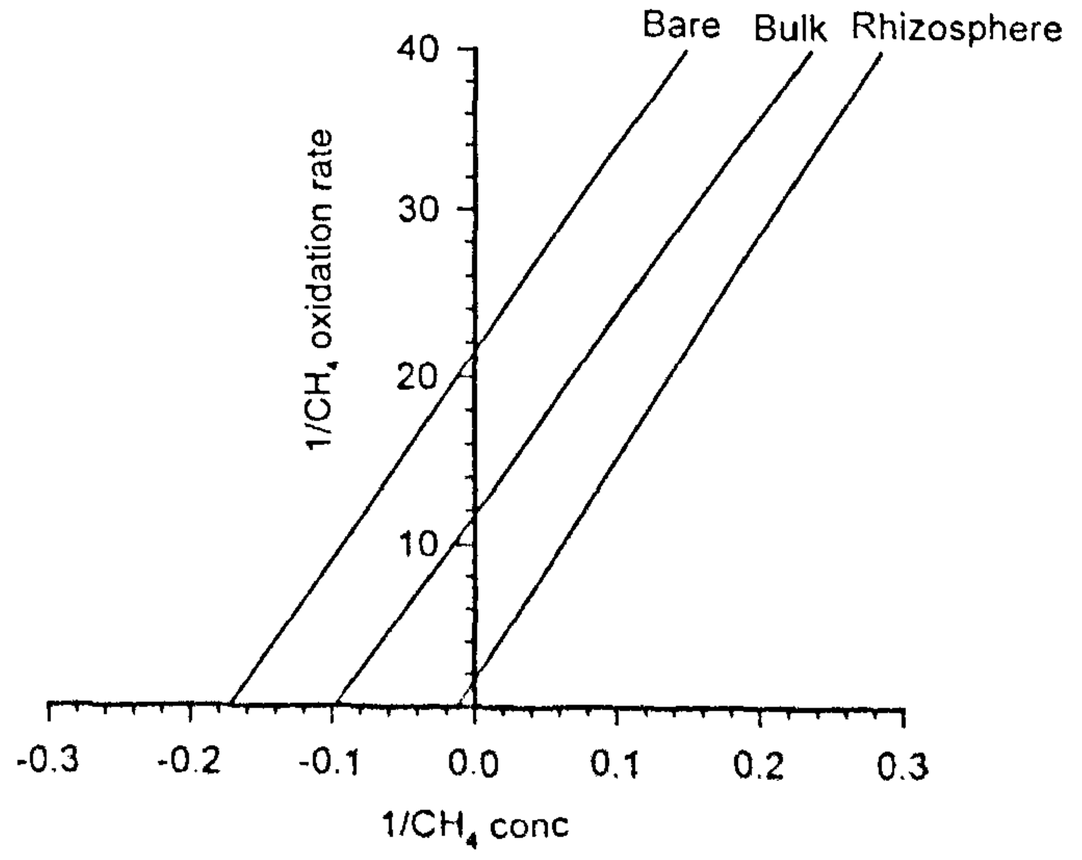

Figure 2. Lineweaver–Burk plots for CH_4 uptake by soil samples. The intercepts and slopes were: bare soil, 21.4816 and 124.835; bulk soil, 11.7568 and 120.0; and rhizosphere soil, 1.6059 and 134.908. r^2 values varied between 0.997 and 0.999. CH_4 concentration is in µg g^{-1} soil; CH_4 oxidation rate is in µg h^{-1} g^{-1} soil.

The direct effect is related to the inhibitory effect of ammonium on MOB and the indirect effect is caused by changing nitrogen turnover[20].

We conclude that rice rhizosphere is a major microsite which supports a large population of MOB due to greater O_2 availability and lower NH_4^+-N concentration, and contributes substantially to the capacity of the soil for CH_4 uptake, leading to a predictable spatial pattern in CH_4 sink strength within the dryland rice ecosystem.

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Somatic embryogenesis and plantlet regeneration from leaf explants of *Sapindus mukorossi* Gaertn.: A soapnut tree

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Somatic embryogenesis and plantlet formation were achieved from leaf and leaf-derived callus culture of *Sapindus mukorossi* Gaertn. Callus subcultured on MS medium containing lower levels of kinetin (0.2–0.5 mg l^{-1}) and higher levels of potassium nitrate (2900 mg l^{-1}) resulted in embryogenic callus often with globular embryos. Rapid and enhanced rate of embryo formation was achieved on transfer of embryogenic callus to an embryo proliferation medium containing kinetin (0.5 mg l^{-1}) and a different source of nitrogen with or without gibberellic acid (0.2 mg l^{-1}). Increased level of gibberellic acid restricted embryo multiplication and led to embryo maturation. Use of kinetin (2.0 mg l^{-1}) along with ammonium chloride (50 mg l^{-1}) and coconut milk, 10% (v/v) in the medium proved to be effective for embryo germination and plantlet formation. This report describes the protocol for somatic embryogenesis and plant regeneration from leaf explant of *S. mukorossi* without any exogenous supply of auxin.

SAPINDUS mukorossi Gaertn. is an important soapnut tropical tree under the family Sapindaceae. It produces soapnut of commerce due to the presence of 15–20% of triterpenoid saponins[1–3]. The fruit is used as a source of mild detergent and in preparation of herbal shampoos in many tropical and sub-tropical countries throughout south-east Asia[4,5]. It has also spermicidal activity in human and rat semen[6]. Tissue culture studies in woody tree species are gaining much significance to develop rapid and large-scale propagation of true-to-type elite clones and also as a prerequisite for the successful application of advances made through techniques such as

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