

8. Matsumoto, K. and Lynch-Stieglitz, J., Similar glacial and Holocene deep water circulation inferred from southeast Pacific benthic foraminiferal carbon isotope composition. *Paleoceanography*, 1999, **14**, 149–163.
9. Ninnemann, U. S. and Charles, C. D., Regional differences in Quaternary sub antarctic nutrient cycling: Link to intermediate and deep water ventilation. *Paleoceanography*, 1997, **12**, 560–567.
10. Oppo, D. W. and Horowitz, M., Glacial deep water geometry: South Atlantic benthic foraminiferal Cd/Ca and  $\delta^{13}\text{C}$  evidence. *Paleoceanography*, 2000, **15**, 147–160.
11. Charles, C. D. and Fairbanks, R. G., Glacial to interglacial changes in the isotopic gradients of Southern Ocean surface waters. In *Geological History of the Polar Oceans: Arctic Versus Antarctic* (eds Bleil, U. and Thiede, J.), Kluwer, Massachusetts, 1990, pp. 519–538.
12. Belkin, I. M. and Gordon, A. L., Southern Ocean fronts from the Greenwich meridian to Tasmania. *J. Geophys. Res.*, 1996, **101**, 3675–3696.
13. Gordon, A. L., Molinelli, E. and Barker, T., Large scale relative dynamic topography of the Southern Ocean. *J. Geophys. Res.*, 1978, **83**, 3023–3032.
14. Deacon, G. E. R., A general account of the hydrology of the Southern Atlantic Ocean. *Discovery Rep.*, 1933, **7**, 71–238.
15. Deacon, G. E. R., The hydrology of the Southern Ocean. *Discovery Rep.*, 1937, **15**, 1–24.
16. Hofmann, E. E., The large-scale horizontal structure of the Antarctic circumpolar current from FGGE drifters. *J. Geophys. Res.*, 1985, **90**, 7087–7097.
17. Orsi, A. H., Whitworth III, T. and Nowlin Jr., W. D., On the meridional extent and fronts of the Antarctic Circumpolar Current. *Deep Sea Res.*, 1995, **1**, 641–673.
18. Nowlin Jr., W. D. and Clifford, M., The kinematic and thermohaline zonation of the Antarctic Circumpolar Current at Drake Passage. *J. Mar. Res.*, 1982, **40**, 481–507.
19. Whitworth, T., Zonation and geostrophic flow of the Antarctic Circumpolar Current at Drake Passage. *Deep Sea Res.*, 1980, **A**, 497–507.
20. Trenberth, K. E., Large, W. G. and Olson, J. G., The mean annual cycle in global ocean wind stress. *J. Phys. Oceanogr.*, 1990, **30**, 1742–1760.
21. Deacon, G. E. R., Physical and biological zonation in the Southern Ocean. *Deep Sea Res.*, 1982, **29**, 1–15.
22. Barker, P. F. and Thoma, E., Origin, signature and palaeoclimatic influence of the Antarctic Circumpolar current. *Earth-Sci. Rev.*, 2004, **66**, 143–162.

ACKNOWLEDGEMENTS. We thank Dr Harsh K. Gupta, Former Secretary, DOD for his personal interest in launching the pilot expedition to the Southern Ocean. We also thank Officials at the Department of Ocean Development, New Delhi and the National Centre for Antarctic and Ocean Research, Goa for their untiring efforts in making this long awaited programme a reality. Special thanks are also due to all participating organizations and their respective scientists who have very kindly provided inputs for the present paper. Captain, officers and crew of PESO are acknowledged for their constant support during the collection of samples.

Received 1 April 2005; revised accepted 3 December 2005

## Marine and estuarine methylotrophs: their abundance, activity and identity

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**Methanotrophs were up to 1 and 0.65% of the total counts in estuarine waters and offshore sediments respectively. Experimental tests on methanol utilization showed that the estuarine isolates grew best at 4% methanol whereas offshore ones grew at 5% at an optimum pH of 6 or 7. Methanol, when used as an additional carbon source, in the presence of nutrient broth concentration ranging from 0.08 to 0.4%, enhanced growth by 129% and respiration by 177% in estuarine isolates. Biochemical and physiological characteristics showed that estuarine methylotrophs exhibited taxonomic affinities to *Pseudomonas* I or II sp. The offshore genera were more varied and belonged to *Flavobacterium* and *Pseudomonas* I or II sp. The abundance, activity and identity suggest that these physiological groups could be widespread and therefore could perhaps contribute significantly to the changes in  $\text{C}_1$  compounds and even their derivatives in marine and estuarine environments.**

**Keywords:** Adaptation, estuarine, methylotrophs, marine, methanol.

METHYLOTROPHIC bacteria (MTB) are obligate aerobic microorganisms recognized by their ability to grow on carbon compounds more reduced than  $\text{CO}_2$ , without any C–C bonds. They are even able to assimilate compounds such as HCHO or a mixture of HCHO and  $\text{CO}_2$ . MTB capable of oxidizing methane are methanotrophs (MOB). They play an important role in the geochemical cycling of methane and its derivatives. The oxidation of methane can have major implications on the structure of food webs and climate, especially in the current global scenario. Hence, a study on their ecology would be pertinent to understand the dynamics of methane and methane-derived compounds, especially in marine and estuarine systems.

Though much work has been carried out on the molecular<sup>1–3</sup> and taxonomic aspects<sup>4</sup> of methanotrophs, the study is either restricted to lacustrine environment<sup>5,6</sup> or terrestrial regions<sup>7–9</sup>. Work on the marine environment is limited<sup>10</sup>. Hence, the present study assesses the retrievable abundance and distribution of methylotrophs and methanotrophs. It also examines the activity of methylotrophs from estuarine beach and offshore regions.

Sampling was carried out during low tide in September, representing the end of the southwest monsoon season at Dona Paula beach (15°27'N, 73°48'E), a sheltered

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beach in North Goa situated at the confluence of the Mandovi and Zuari estuaries. Three adjacent sediment cores were collected using hand-corers. The cores were sectioned into 0–5, 5–10 and 10–13 cm. Water and core samples were transported to the laboratory in iceboxes and analysed within 2–3 h of collection.

Water samples were collected into sterile polypropylene bottles from which 5 ml was transferred into vials and fixed with 250  $\mu$ l buffered formalin. Total bacterial populations were estimated using acridine orange direct counts (AODC) method<sup>11</sup>. For sediment samples, approximately 1 g was transferred to a 15 ml centrifuge tube filled with 9 ml autoclaved filtered sea water, vortexed for 5 min and allowed to settle for 1 min. Five millilitre of supernatant was transferred into vials and fixed with 250  $\mu$ l buffered formalin. One millilitre of the fixed sample was mixed with acridine orange (final concentration 0.01% w/v) for 5 min. The contents were then filtered onto 0.22  $\mu$ m pore-size black-stained Nuclepore filter paper. Bacterial counts were made at 100X with an epifluorescence microscope (BH) using 515-barrier filter.

Isolation of methylotrophs and methanotrophs was carried out onto ATCC #1306 medium<sup>12</sup>. The medium contained [g l<sup>-1</sup> sea water (50%) at pH 6.8]: MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.0; CaCl<sub>2</sub>·6H<sub>2</sub>O, 0.2; FeNH<sub>4</sub>EDTA, 0.004; KNO<sub>3</sub>, 1.0; KH<sub>2</sub>PO<sub>4</sub>, 0.272; Na<sub>2</sub>HPO<sub>4</sub>·12 H<sub>2</sub>O, 0.717 and 0.5 ml trace element solution. Plates were solidified with 1.8% of purified bacto agar. Approximately 5 g of sediment sample was suspended in a conical flask containing 45 ml of sterile sea water, vigorously shaken for 1 min and diluted up to 10<sup>6</sup>. Suitable aliquots (50–100  $\mu$ l) from 10<sup>2</sup> to 10<sup>6</sup> dilutions were surface-plated onto ATCC #1306 medium. The plates were incubated with methane in Gas Pak jars in the dark for 3–4 weeks and checked for bacterial growth against a control. For the isolation of methylotrophs the procedure was the same, except that methanol was supplied in the vapour phase<sup>13</sup> from a petri plate placed at the bottom of Gas Pak jar. Growth was recorded for 7 to 10 days period against a control. The counts were expressed as CFU g<sup>-1</sup> dry sediment or CFU ml<sup>-1</sup>. Plates were divided into sectors and a sector was randomly chosen and all CFU were isolated, checked for purity and used for experiments and characterization. Colonies of similar morphotypes were isolated (in replicates of 4 to 5). Thus six methylotrophic isolates each ES6, ES7, ES31, OF401, OF504 and OF507 from estuarine and offshore regions represent about 30 original isolates which were identified according to Gerhardt<sup>14</sup>.

Adaptation to aqueous methanol was achieved by sequential transfer of cultures into media with progressively higher concentration of methanol (0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0% v/v). Subsequently, experiments were carried out with 4% methanol for estuarine and 5% for offshore isolates. Experiments were carried out to measure growth and respiration under varying methanol, organic carbon concentrations, and pH. Growth was estimated at definite

intervals by direct cell counts on a haemocytometer. Simultaneously, respiration was monitored in terms of formazan production from TTC<sup>15,16</sup>. Cultures were incubated at (30  $\pm$  2°C) for a period of 8 days.

To test the effect of organic carbon on growth and respiration, isolates were grown in mineral media containing methanol supplemented with various concentrations (0.08, 0.16 and 0.4%) of nutrient broth.

To test the effect of pH on growth and respiration, isolates were grown in mineral media containing methanol adjusted to various pH values, viz. 6–9. Control tubes without methanol were included.

Microbial Adhesion to Hydrocarbons (MATH) Assay was carried out to assess the ability of the isolates to utilize hydrocarbons, as outlined by Rossenberg<sup>17</sup>. The assay was carried out as follows: To a thick suspension of a day old culture, 4 ml of phosphate buffer (pH 8) was added and the same was vortexed and its initial OD was adjusted to 0.2 at 550 nm. To this, 0.5 ml of *n*-undecane was added and vortexed for 2 min. This was allowed to stand at room temperature for 20 min for phase separation. Turbidity of the lower aqueous phase was measured again and fraction of adherence calculated.

Fraction of cell adherence is defined as the ratio of the difference between the initial and final turbidity over the initial value.

$$\text{Fraction of adherence} = (A - C)/A,$$

where *A* is the initial turbidity and *C* the final turbidity.

Both water and sediment from the estuarine and offshore regions were tested for the presence of methylotrophs and methanotrophs. Culturable methanotrophs ranged between 0.13 and 6.12  $\times$  10<sup>3</sup> CFU g<sup>-1</sup> estuarine sediment and between 0.17 and 0.85  $\times$  10<sup>3</sup> CFU g<sup>-1</sup> offshore sediment. However, methanotrophs from water were two orders more at 807.0  $\times$  10<sup>3</sup> CFU ml<sup>-1</sup> in the estuary than in the sea, where they ranged between 10<sup>1</sup> and 10<sup>3</sup> ml<sup>-1</sup>. However, Faria *et al.*<sup>18</sup> reported that the MOB could range higher from 10<sup>4</sup> to 10<sup>6</sup> g<sup>-1</sup> much later in the year (post-monsoon) in offshore sediments of the Arabian Sea and these were over three orders higher than the present values. Yet another comparison showed that abundance of methanotrophs in this study is one order less than the values reported by Takeuchi *et al.*<sup>19</sup> from sediment using MPN technique. The difference in abundance could be partly due to differences in ecosystems and also differences in the techniques employed. Like methanotrophs, methylotrophs retrieved on methanol-containing media varied from 0.63 to 66.9  $\times$  10<sup>3</sup> CFU g<sup>-1</sup> estuarine sediment, but were below detection limits offshore. Methylotrophic abundance was higher in the estuarine waters at 49.60  $\times$  10<sup>3</sup> CFU ml<sup>-1</sup> than offshore (Table 1). Ross *et al.*<sup>20</sup> reported higher values for methylotrophic bacterioplankton that varied between 0.6 and 1.2  $\times$  10<sup>6</sup> ml<sup>-1</sup> in the winter of 1994, and 0.8 and 5.5  $\times$  10<sup>6</sup> ml<sup>-1</sup> in the summer of 1994–95 in the floodplain lake in northeastern Victoria, Australia

## RESEARCH COMMUNICATIONS

**Table 1.** Comparison of abundance of methylotrophic and methanotrophic bacteria with other studies

Source	Number of MTB	Number of MOB	Method	Reference
Marine sediment ( $\text{g}^{-1}$ )				
Arabian Sea sediment–January to February 2002	BDL	$0.17\text{--}0.85 \times 10^3$	Surface plating	Present study
Arabian Sea sediment–November 2002	ND	$10^4\text{--}10^6$		Faria <i>et al.</i> <sup>18</sup>
Water ( $\text{ml}^{-1}$ )	$0.08\text{--}4.00 \times 10^3$	$0.08\text{--}7.3 \times 10^3$		Present study
Estuarine sediment	$0.63\text{--}66.9 \times 10^3$	$0.13\text{--}6.12 \times 10^3$		
Water	$49.60 \times 10^3$	$807.00 \times 10^3$		
Others				
Aquifer sediment	–	$10^4$	MPN	Takeuchi <i>et al.</i> <sup>20</sup>
Floodplain lake water				
1994 Winter	$0.6\text{--}1.2 \times 10^6$	–	16S rRNA probes	Ross <i>et al.</i> <sup>21</sup>
1994–95 summer	$0.8\text{--}5.5 \times 10^6$			

BDL, Below detection limit; ND, Not done; MTB, Methylotrophic bacteria; MOB, Methane-oxidizing bacteria; MPN, Most probable number technique.

**Table 2.** Total counts of bacteria from estuarine and offshore waters

Total counts ( $\text{ml}^{-1}/\text{g}^{-1}$ )	Estuarine		Offshore	
	Water	Sediment	Water	Sediment
	$\times 10^7$	$\times 10^5$	$\times 10^7$	$\times 10^8$
	1.8–1.9	2.4–1.3	1.15–8.19	0.09–1.18

(Table 1). Thus, our study shows that methylotrophs are two orders less than the estimates made by Ross *et al.*<sup>20</sup> using 16S rRNA probes. Irrespective of the region, water recorded higher abundance than sediment in the present study. The present study shows that the methylotrophs and methanotrophs were generally more abundant in estuaries with water showing a higher concentration than sediment, suggesting that the substrates or substrate producers for these organisms could be more abundant in estuarine waters. Particles in estuarine waters could harbour higher number of fermentative and methanogenic bacteria which could provide these substrates.

The total bacterial counts in water were in the order of  $10^7 \text{ ml}^{-1}$ . However, in the estuarine beach and offshore sediments, they were in the order of  $10^5$  and  $10^8 \text{ g}^{-1}$  respectively (Table 2). Takeuchi *et al.*<sup>19</sup> reported total counts in the order of  $10^4 \text{ g}^{-1}$  dry sediment, which is lower than that observed in the present study. Methanotrophs formed up to 1% of total counts in estuarine water and 0.005% in sediment. The contribution of these forms amounted to 0.65 and 0.03% of the total counts in offshore sediments and water respectively. These values are congruent with those reported by other authors. Gilbert and Frenzel<sup>21</sup> reported that MOB estimated in a planted paddy soil accounted for 1% of total counts (direct counts of total bacteria vs MPN of MOB). MOB are also known to account for the same number as aerobic heterotrophic bacteria<sup>22</sup>. However, Vecherskaya *et al.*<sup>23</sup> reported that MOB estimated by immunofluorescence formed 1–23% of total counts in peat soils from Siberian tundra.

Takeuchi *et al.*<sup>19</sup> reported high percentages of 15.71, 4.78 and 5.08 for three aquifers from TCE-contaminated site in Chikura, Chiba, Japan. The higher percentages of MOB reported by these authors could perhaps be attributed not only to the different ecosystems, but also to the lower total bacterial counts obtained from that system. MPN methods are known to yield higher numbers. Dubey *et al.*<sup>24</sup> reported that most quantification of MOB population size relies on MPN methods. Frenzel<sup>22</sup> highlights some of the limitations of this method. These include microcolonies that could be counted instead of single cells, the medium could be selective for certain strains, and cells may be in an unculturable state.

Despite the widely reported toxicity of methanol to obligate methylotrophs<sup>25–27</sup>, growth on methanol at high concentrations is clearly possible for *Methylocystis parvus* OBBP up to 4% w/v<sup>28</sup>, *Methylococcus* NCIB 11083 up to 0.2%, v/v<sup>28</sup> and *Methylosinus trichosporium* OB3b up to 4% v/v<sup>29</sup>. In the present study, some of the estuarine isolates grew and respired at 4 or 5% v/v concentration of methanol. The mechanism by which these organisms adapt to growth on methanol at high concentrations is unknown, but may reflect physiological adaptations of the population to the substrate or the selection of a mutant to either methanol or formaldehyde<sup>29</sup>. Loss of this ability of methanol-grown organisms on methane or substrate analogues has been reported for bacterium B6 (ref. 30) and for three other methylotrophs<sup>31</sup>. In contrast, other workers have demonstrated the retention of methane-oxidizing activity in type I methylotrophs<sup>25,28</sup>. Results of Best and

Higgins<sup>13</sup> were in contrast with those of Hou *et al.*<sup>31</sup> for the same strain of *M. trichosporium*. In the present study the six isolates also displayed retention of such methanol-oxidizing activity, suggesting that the enzyme is either constitutive or induced by methanol.

Many methylotrophs are facultative or restricted facultative, that grow on sugars, fatty acids, amino acids, inorganic substrates, and complex media as well as one-carbon compounds. However, in the present study, methylotrophs could utilize either methane or methanol effectively for their growth in the presence of low concentration of nutrient broth (0.1%). It has been reported that some cultures utilizing methanol grow better on heterotrophic substrates<sup>30,32</sup>, and it is suggested that methanotrophs with heterotrophic tendencies would be more widespread than strict autotrophic ones. According to Griffiths *et al.*<sup>33</sup>, there is extensive documentation that methane-utilizing bacteria can utilize a large number of organic compounds that include not only simple hydrocarbons, but also more complex organic molecules<sup>33-36</sup>.

Experiments with isolates showed that maximum growth and respiration were recorded after a period of 8 days for all methylotrophs with the exception of OF507, which showed maximum growth and respiration after 6 days. With increasing organic carbon from 0.08 to 0.4%, enhanced growth (129%) and respiration (177%) were recorded for estuarine isolates. However, there was a 17% decrease in growth and 396% increase in respiration for their marine counterparts.

The optimum pH recorded for the estuarine and offshore isolates was 6 or 7. On an average, growth of offshore isolates was 199% of the control at pH 6 or 7, and was better than its estuarine counterparts, which was only

166%. Respiration was stimulated by 80 and 70% of the control for offshore and estuarine methylotrophs respectively. At 4 and 5% methanol concentration for estuarine and offshore respectively, their ability to grow at different pH values, and organic carbon concentration (0.08–0.4%) showed that these isolates have an optimal pH at 6 or 7. Borne *et al.*<sup>37</sup> reported methane oxidation from pH 3.5 to 8.0. However, Dedysh *et al.*<sup>38</sup> reported isolation of methanotrophs from *Sphagnum* peat bogs incapable of growth at pH values below 5.0. Calhoun and King<sup>39</sup> report isolation of methanotrophs from freshwater macrophytes at pH values between 6 and 7. Irrespective of the region, isolates could grow in a broad range of pH values with an optimum at 6 or 7 in this study.

Molar growth yield on methanol was  $2 \times 10^{10}$  cells ( $\pm 3.4$ ) and  $4 \times 10^{10}$  cells ( $\pm 1.2$ ) for offshore and estuarine methylotrophs respectively. The molar growth yield constant on methanol for offshore methylotrophs was 0.02 and for estuarine methylotrophs 0.04. Offshore forms are lower in yield, but higher in activity (Tables 3 and 4). Faria *et al.*<sup>18</sup> also reported that offshore MOB are significantly higher in abundance than near-shore ones ( $P > 0.001$ ). Thus it is suggested that offshore MOB are not only more abundant in certain seasons, but also could be higher in activity.

MATH assay, a cell-surface hydrophobicity test indicates that growth of four of the six methylotrophs under the conditions described displayed a hydrophobic character, as evidenced by their association with the immiscible *n*-undecane phase (Table 4). The fraction of adherence for culture OF504 was high, due to its hydrocarbonoclastic property. Raiker *et al.*<sup>40</sup> observed the fraction of adherence for thraustochytrids to be high and ranging between 0.2 and 0.42. The fraction of adherence was however less for OF507 (0.0098), ES7 (0.178) and closer to that of OF401 (0.35).

The high fraction of adherence for OF401 (0.445) could be due to its hydrocarbonoclastic property. De Souza *et al.*<sup>41</sup> reported a high fraction of adherence (0.4–0.5) for culture P43, a phosphate-solubilizing bacteria. In the present study cultures ES6 and ES31 were hydrophilic, exhibiting an adherence of –0.23 and –0.055 respectively. The hydrophilic nature apparently reflects the presence of vegetative cells<sup>42</sup> and the probable presence of poly *b* hydroxybutyrate granules<sup>43</sup>. Hydrophobic property exhibited by methylotrophic bacteria makes them ideal candidates for the bio-remediation of hazardous environmental wastes like trichloroethylene<sup>44</sup> and other xenobiotics.

Phenotypic characterization based on key biochemical (catalase, oxidase and modified oxidative fermentative tests, ability to use methane, methanol and *n*-undecane) and morphological traits (colony morphology, Gram stain, motility, size, etc.) showed that 33% of the offshore isolates and all the estuarine methylotrophs showed taxonomic affinities to *Pseudomonas* I and II sp. The offshore genera were more varied and belonged to *Flavobacterium*

**Table 3.** Molar growth yield constant (*k*) of estuarine and offshore MTB

	Methanol		
	4%		5%
	Estuarine methylotrophs Ps. sp ( <i>n</i> = 3)	Offshore methylotrophs Ps. sp ( <i>n</i> = 2)	Fl sp ( <i>n</i> = 1)
	0.041	0.024	0.018
Avg.	0.04		0.02

**Table 4.** MATH assay

Culture	Initial turbidity (A)	Final turbidity (C)	Fraction of adherence (A–C)
ES6	0.200	0.246	–0.230
ES7	0.200	0.170	0.178
ES31	0.206	0.211	–0.055
OF401	0.203	0.133	0.350
OF504	0.200	0.111	0.445
OF507	0.203	0.201	0.0098

and *Pseudomonas* I and II sp. As these taxonomical groups are widespread in the marine environment and as the abundance of methylotrophs was as high as 1%, it is speculated that they could contribute significantly to the oxidation of methanol and its derivatives in nature. These groups could perhaps also participate in the oxidation of methane in the marine and estuarine environments. Experiments are underway to examine their methane-oxidizing activity in estuarine and marine sediments under simulated *in situ* conditions.

1. Eller, G. and Frenzel, P., Changes in activity and community structure of methane-oxidizing bacteria over the growth period of rice. *Appl. Environ. Microbiol.*, 2001, **67**, 2395–2403.
2. Bodelier, P. L. E., Roslev, P., Henckel, T. and Frenzel, P., Stimulation by ammonium based fertilizers of methane oxidation in soil around rice roots. *Nature*, 2000, **403**, 421–424.
3. Heyer, J., Galchenko, V. F. and Dunfield, P., Molecular phylogeny of type II methane-oxidizing bacteria isolated from various environments. *Microbiology*, 2002, **148**, 2831–2846.
4. Kaluzhnaya, M. *et al.*, Taxonomic characterization of new alkaliphilic and alkalitolerant methanotrophs from soda lakes of the Southeastern Transbaikalian region and description of *Methylomicrobium buryatense* sp. nov. *Syst. Appl. Microbiol.*, 2001, **24**, 166–176.
5. Linton, J. D. and Vokes, J., Growth of the methane-utilizing bacterium *Methylococcus* NCIB 11083 in mineral salts medium with methanol as the sole source of carbon. *FEMS Microbiol. Lett.*, 1978, **4**, 125–128.
6. Patel, R. N., Hou, C. T., Derelanko, P. and Felix, A., Microbial oxidation of methane and methanol: Isolation of methane utilizing bacteria and characterization of a facultative methane-utilizing isolate. *J. Bacteriol.*, 1978, **136**, 352–358.
7. Dubey, S. K., Sinha, A. S. K. and Singh, J. S., Spatial variation in the capacity of soil for methane uptake and population size of methane-oxidizing bacteria in dryland rice agriculture. *Curr. Sci.*, 2000, **78**, 617–620.
8. Dubey, S. K. and Singh, J. S., Plant-induced spatial variation in the size of methanotrophic population in dryland and flooded rice agroecosystems. *Nutr. Cycl. Agroecosyst.*, 2001, **59**, 161–167.
9. Dubey, S. K., Sinha, A. S. K. and Singh, J. S., Differential inhibition of methane oxidation in bare, bulk and rhizosphere soils of dryland rice field by nitrogen fertilizers. *Basic Appl. Ecol.*, 2002, **3**, 347–355.
10. Holmes, A. J., Owens, N. J. P. and Murrell, J. C., Molecular analysis of enrichment cultures of marine methane oxidizing bacteria. *J. Exp. Mar. Biol. Ecol.*, 1996, **203**, 27–38.
11. Hobbie, J. E., Daley, R. J. and Jaspers, S., Use of nucleopore filters for country bacterial by fluorescence microbiology. *Appl. Environ. Microbiol.*, 1977, **33**, 1225–1220.
12. Gherna, R., Pienta, P. and Cote, R., *American Type Culture Collection, Catalog of Bacteria and Bacteriophages*, Rockville, Maryland, 1989, 17th edn.
13. Best, D. J. and Higgins, I. J., Methane oxidizing activity and membrane morphology in a methanol grown obligate methanotroph, *Methylosinus trichosporium* OB3b. *J. Gen. Microbiol.*, 1981, **125**, 73–84.
14. Gerhardt, P. (ed.), *Manual of Methods for General Bacteriology*, American Society of Microbiology, Washington DC, 1981.
15. Kumar, P. and Tarafdar, J. C., 2,3,5-Triphenyltetrazolium chloride (TTC) as electron acceptor of culturable soil bacteria, fungi and actinomycetes. *Biol. Fertil. Soils*, 2003, **38**, 186–189.
16. Lenhard, G., Die Dehydrogenaseaktivität des Bodens als Mass für die Mikroorganismenaktivität II Boden. *Z. Pflanzenernaehr Deung Bodenkd.*, 1956, **73**, 1–11.
17. Rossenberg, M., Basic and applied aspects of microbial adhesion at the hydrocarbon: water interface. *Crit. Rev. Microbiol.*, 1991, **18**, 159–173.
18. Faria, D., De Souza, M. J. D., Nair, S., Loka Bharathi, P. A., Biswas, G. and Anbazhagan, K., Bacterial indicators of methane variability in the Arabian Sea, West coast of India (communicated).
19. Takeuchi, M., Nanba, K., Iwanoto, H., Nirei, H., Kazaoka, O. and Furuya, K., Distribution and seasonal variation of methanotrophs in trichloroethylene contaminated groundwater. *Water Res.*, 2000, **34**, 3717–3724.
20. Ross, J. L., Boon, P. I., Ford, P. and Hart, B. T., Detection and quantification with 16S rRNA probes of planktonic methylotrophic bacteria in a floodplain lake. *Microb. Ecol.*, 1997, **34**, 97–108.
21. Gilbert, B. and Frenzel, P., Rice roots and CH<sub>4</sub> oxidation: The activity of bacteria, their distribution and the microenvironment. *Soil Biol. Biochem.*, 1998, **30**, 1903–1916.
22. Frenzel, P., Plant associated methane oxidation in rice fields and wetlands. *Adv. Microbiol.*, 2000, **16**, 85–114.
23. Vecherskaya, M. S., Galchenko, V. F., Sokolova, E. N. and Samarkin, V. A., Activity and species composition of aerobic methanotrophic communities in tundra soils. *Cum. Microbiol.*, 1993, **27**, 181–184.
24. Dubey, S. K., Padmanabhan, P., Purohit, H. J. and Upadhyay, S. N., Tracking of methanotrophs and diversity in paddy soil: A molecular approach. *Curr. Sci.*, 2003, **10**, 92–95.
25. Leadbetter, E. R. and Foster, J. W., Studies on some methane-utilizing bacteria. *Arch. Mikrobiol.*, 1958, **30**, 91–118.
26. Stocks, P. K. and McCleskey, C. S., Morphology and physiology of *Methanomonas methanooxidans*. *J. Bacteriol.*, 1964, **88**, 1071–1077.
27. Whittenbury, R., Phillips, K. C. and Wilkinson, J. F., Enrichment, isolation and some properties of methane-utilizing bacteria. *J. Gen. Microbiol.*, 1970, **61**, 205–218.
28. Hou, C. T., Laskin, A. I. and Patel, R., Growth and polysaccharide production by *Methylocystis parvus* OBBP on methanol. *Appl. Environ. Microbiol.*, 1979, **37**, 800–804.
29. Oliver, J. D., Taxonomic scheme for the identification of marine bacteria. *Deep Sea Res.*, 1982, **29**, 795–798.
30. Anthony, C., The microbial metabolism of C1 compounds. The cytochromes of *Pseudomonas* AM1. *Biochem. J.*, 1975, **146**, 289–298.
31. Hou, C. T., Patel, R., Laskin, A. I. and Barnabe, N., Microbial oxidation of gaseous hydrocarbons: epoxidation of C<sub>2</sub>–C<sub>4n</sub>-alkenes by methylotrophic bacteria. *Appl. Environ. Microbiol.*, 1979, **38**, 127–134.
32. Colby, J. and Dalton, H., Characterization of the second prosthetic group of the flacoenzyme NADH-acceptor reductase (component C) of the methane monooxygenase from *Methylococcus capsulatus* (Bath). *Biochem. J.*, 1979, **177**, 903–908.
33. Griffiths, R. P., Caldwell, B. A., Cline, J. D., Broich, W. A. and Morita, R. Y., Field observations of methane concentrations and oxidation rates in the southeastern Bering Sea. *Appl. Environ. Microbiol.*, 1982, **44**, 435–446.
34. Higgins, I. J., Hammond, R. C., Sarialani, F. S., Best, D., Davies, M. M., Tryhorn, S. E. and Taylor, F., Biotransformations of hydrocarbons and related compounds by whole – organism suspensions of methane-grown *Methylosinus trichosporium* OB3b. *Biochem. Biophys. Res. Commun.*, 1979, **89**, 671–677.
35. Higgins, I. J., Best, D. J. and Hammond, R. C., New findings in methane-utilizing bacteria highlight their importance in the biosphere and their commercial potential. *Nature*, 1980, **286**, 561–564.
36. Patt, T. E., Cole, G. C., Bland, J. and Hanson, R. S., Isolation and characterization of bacteria that grow on CH<sub>4</sub> and organic compounds as Sole sources of carbon and energy. *J. Bacteriol.*, 1974, **120**, 55–964.

37. Borne, M., Dorr, H. and Levin, I., Methane consumption in aerated soils of the temperate zone. *Tellus*, 1990, **42**, 2–8.
38. Dedysh, S. N., Panikov, N. S. and Tiedje, J. M., Acidophilic methanotrophic communities from *Sphagnum* peat bogs. *Appl. Environ. Microbiol.*, 1998, **64**, 922–929.
39. Calhoun, A. and King, G. M., Characterization of root-associated methanotrophs from three freshwater macrophytes: *Pontederia cordata*, *Sparganium eurycarpum*, and *Sagittaria latifolia*. *Appl. Environ. Microbiol.*, 1998, **64**, 1099–1105.
40. Raiker, M. T., Raghukumar, S., Vani, V., David, J. J. and Chandramohan, D., Thraustochytrid protists degrade hydrocarbons. *Indian J. Mar. Sci.*, 2001, **30**, 139–145.
41. De Souza, M. J. B. D., Nair, S., David, J. J. and Chandramohan, D., Crude oil degradation by phosphate-solubilizing bacteria. *J. Mar. Biotechnol.*, 1996, **4**, 91–95.
42. Jenkins, M. B., Chen, J. H. D., Kadner, J. and Lion, L. W., Methanotrophic bacteria and facilitated transport of pollutants in aquifer material. *Appl. Environ. Microbiol.*, 1994, **60**, 3491–3498.
43. Glazer, A. N., Roger Yate Stanier, 1916–1982: a transcendent journey. *Int. Microbiol.*, 2001, **4**, 59–66.
44. Jahng, D., Kim, C. and Wood, T. K., Trichloroethylene degradation using recombinant bacteria expressing the soluble methane-monooxygenase from *Methylosinus trichosporium* OB3b. Abstr. Pap. Am. Chem. Soc., 1995, 209 Meet., Pt. 1, BIOT097.

ACKNOWLEDGEMENTS. We thank Dr Chandramohan, Former Deputy Director, HOD, BOD and Dr E. Desa, Ex Director NIO, Goa for providing the opportunity to carry out the work. D.F. also thanks Prof. G. N. Nayak, HOD Marine Sciences, and Goa University for providing the necessary facilities to carry out the dissertation. An anonymous referee helped improve the contents of the text. This is NIO contribution no. 4080.

Received 2 July 2005; revised accepted 5 December 2005

## Historic submergence and tsunami destruction of Nancowrie, Kamorta, Katchall and Trinket Islands of Nicobar district: Consequences of 26 December 2004 Sumatra–Andaman earthquake

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**The 26 December 2004 Sumatra–Andaman earthquake is one of the largest plate-boundary earthquakes in the recent seismic history of the world. This earthquake has also generated the greatest tsunami run-up and coastal devastation ever recorded. Our field study at four major islands of Nancowrie group of Nicobar District in Andaman and Nicobar archipelago has revealed that the islands are vertically subsided by 1.0–**

**1.75 m with the submergence of coastal land area by thousands of square kilometers. It is also suggested based on our field observations that societal and socio-economic rejuvenation of the islands will need resurvey of entire topography of the islands, coastal bathymetry mapping and identification of newly developed ecological regimes. We have also prepared maps of the coastal submergence for these islands using field observations and remote sensing in this paper. Based on the present field study and geodetic studies by other workers, differential tilting of the Andaman micro-plate is also inferred.**

**Keywords:** Coastal submergence, Nancowrie group, Nicobar Islands, tsunami.

THE Sumatra–Andaman earthquake of 26 December 2004 occurred on 6:29 IST (0.58 UTC) at the subduction plate boundary where the Indian and Australian plates converge and plunge below the Sunda plate. The  $M_w$  9.3 (revised magnitude) plate boundary earthquake is located at 3.7°N and 95°E off the Sumatra coast near the island of Simuelue with a focal depth<sup>1</sup> of ~15 km. The earthquake is considered as the second largest ever recorded on the globe, and it caused wobbling of the earth's axis<sup>2</sup>. Distribution of aftershocks reveals that the rupture plane is about 1200 km long extending to the north, up to the Andaman and Nicobar Islands<sup>3,4</sup>. Immediate observation by the satellite imageries confirms large-scale subsidence around the epicentral zone and many kilometers north of it, in the Andaman and Nicobar Islands. The present study on preliminary documentation of ground deformation and tsunami effects on the Nancowrie group of islands of Nicobar district, was carried out based on satellite imageries. These imageries provide information on inundation of the islands and site-specific details of subsidence and tsunami run-ups at each location. An attempt is also made to prepare preliminary maps that show the coastal area of subsidence on four islands – Nancowrie, Kamorta, Katchall and Trinket of the Nancowrie group. These inundation maps could be used in future planning of developmental activities in these islands. The coastal villages mentioned later and farm fields on all four islands can be identified as areas likely to be submerged in the future. A similar study on the other islands of Andaman and Nicobar groups could reveal the tectonic behaviour of the Andaman micro-plate.

The Andaman and Nicobar groups of 349 islands, situated in the Bay of Bengal, are separated by the ten-degree channel (Figure 1). The rocks of these islands are believed to have been formed from the sediments scraped off the descending Indian plate interleaved with ophiolites from the ocean floor beneath the Bengal Fan. Detailed geology of the Andamans has been described by Oldham<sup>5</sup> and Tipper<sup>6</sup>. The earliest rocks found in Andamans are Upper Cretaceous clastics with ultramafic and mafic intrusives. A complete succession of Tertiary rocks is found in the Andaman group of islands. Pleistocene sand beds,

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