## Deep-sea benthic foraminifera from gas hydrate-rich zone, Blake Ridge, Northwest Atlantic (ODP Hole 997A)

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Certain species of benthic foraminifera feed on rich bacterial food sources at methane seeps, indicating their potential as proxy for methane fluxes in the geological record. Several of these species have been reported in different methane-rich marine settings and have proved to be good indicators of methane eruptions. The Blake Ridge, located ~350 km off the coast of South Carolina, northwestern Atlantic, is a large drift deposit and a proven gas hydrate field, as is evident by the geochemical anomalies and presence of a bottom simulating reflector. This area thus offers good opportunity to analyse benthic faunal-gas hydrate relationship over different timescales. Our newly generated benthic foraminiferal faunal and published total organic carbon data from Ocean Drilling Program Hole 997A suggest in situ production of methane by bacterial decomposition of organic matter. We suggest that the fluctuating sea level in response to changes in the Northern Hemisphere continental ice volume may have caused the release of methane from the Blake Ridge gas hydrates during the past 3 Ma. We expect the results of this study to help in the exploration of gas hydrates on the continental shelf of India, which archives a thick pile of sediments with high organic carbon content - ideal for gas hydrate formation.

BENTHIC foraminifera are an important component of the marine community and are sensitive to environmental changes.

Over the last three decades, scientists have increased their interest to understand different aspects of benthic foraminifera for palaeoenvironmental reconstructions. The wide geographical and bathymetric distribution, high sensitivity to various ecological factors, extensive morphological diversity, and well-preserved fossil record make them an important tool in palaeoceanography and palaeoclimatology. Some species of benthic foraminifera have been found associated with rich organic carbon content of marine sediments<sup>1-6</sup>. Benthic foraminifera have been found in different methanerich marine settings and have proved to be good indicators of methane releases<sup>7-11</sup>. Some species prefer to feed on rich bacterial food sources at methane seeps, showing their potential as indicators of methane release in the geological record<sup>11</sup>. Some methane-loving taxa include species of Uvigerina, Bolivina, Bulimina, Chilostomella, Globobulimina and Nonionella7-13, which can withstand such stressful conditions. Highly depleted carbon isotopic values in the shells of dead or living benthic and planktic foraminifera and other proxies help in identifying the methane-rich environment. Very high negative excursion (-6 to -2%) of  $\delta^{13}$ C in the shells of Uvigerina, Bolivina and Nonionella have been found related to methane excursions<sup>8-10,13</sup>. In the present study, we have attempted to understand the relation between deep-sea benthic foraminifera, in situ methane formation and methane releases from Ocean Drilling Program (ODP) Hole 997A, Leg 164, Blake Ridge, northwest Atlantic during the latest Miocene-Pleistocene (Figure 1). We propose to test our model based on benthic foraminifera in the marine sediments of the Indian Ocean in identifying gas hydrate horizons in future endeavours.

ODP Hole 997A is located on the crest of the Blake Outer Ridge (31°50.588'N; 75°28.118'W; water depth 2770.1 m), northwest Atlantic, off the east coast of United States of America<sup>14</sup> (Figure 1). Geophysical survey shows a strong bottom simulating reflector (BSR) present between the gas hydrate zone (top) and free gas zone (below), indicating that the supply rate of methane in Hole 997A exceeds the critical value<sup>15</sup>. Using other geochemical and geophysical proxies (temperature, interstitial water, chloride content and electrical resistivity data), it is proved that disseminated gas hydrate occurs throughout the sedimentary section between ~180 and ~450 m below seafloor (mbsf)<sup>14</sup>, which may extend up to ~30 mbsf<sup>16</sup> (Figure 2). Hole 997A consists of a pile of Neogene drift deposits dominated by finegrained nannofossil-bearing hemipelagic sediments<sup>17</sup> accumulated at unusually high rates during the late Miocene (average 7.97 cm/kyr) and Pliocene (average 9.91 cm/kyr), with a substantial drop in sedimentation rate during the Pleistocene (average 4.61 cm/kyr). This hole is presently situated under the profound influence of the northward-flowing Gulf Stream surface current as well as southward-flowing Western Boundary undercurrent, which carried the clastic materials and formed the ridge<sup>18</sup>. The modern lysocline lying between the 4000 and 4350 m water depth is related to the mixing zone of Antarctic Bottom Water and North Atlantic Deep Water in the subtropical northwest Atlantic<sup>19</sup>.

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We analysed 246 samples (at an average interval of 22.22 kyr), each of 10 cm<sup>3</sup> volume, from the latest Miocene to Holocene (5.5 Ma to Recent). Samples were soaked for 12 h in a solution of water and diluted H<sub>2</sub>O<sub>2</sub> (15%) and washed over a 63  $\mu$ m size sieve with a jet of water. We used methylene blue staining solution after each wash to avoid contamination. The washed samples were oven-dried at 50°C. The samples were dry-sieved over a 125  $\mu$ m size sieve for microscopic examination. They were split into suitable aliquots to get 250 to 300 specimens of benthic foraminifera from each sample. Benthic specimens were counted and their relative abundances were calculated. Ages were interpolated for each sample based on the timescale of Okada<sup>20</sup> and updated to that of Berggren *et al.*<sup>21,22</sup>. The relative abundances of some dominant species



**Figure 1.** Map showing location of Ocean Drilling Program sites on the Blake Ridge, northwest Atlantic<sup>14</sup>, drilled during Leg 164.



**Figure 2.** Graph of chlorine concentration in sediments of ODP Hole 997A plotted against depth (metres below seafloor). Gas hydrate zone with depleted chlorine concentration is shown by shaded bar<sup>14</sup>.

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in the hydrate zone.



are plotted in Figure 3 to understand their responses to

We used individual benthic foraminifera species to un-

derstand their behaviour in a methane-rich environment

(gas hydrate zone) at Hole 997A. Bolivina paula (1-23%),

Cibicides kullenbergi (1-36%), Uvigerina hispido-costata

(1-29%) and Uvigerina peregrina (1-34%) are some of

the taxa abundant in the gas hydrate zone at Hole 997A.

B. paula also shows significant peaks in the hydrate-free

upper zone (Figure 3). Pyrgo lucernula is almost absent

hydrocarbon-seep bacterial mats and hydrocarbon vents

in the Gulf of Mexico<sup>7,12</sup>, seep zone at Eel River, northern

California Margin<sup>9</sup>, seep zone of Hydrate Ridge, Oregon<sup>11,13</sup>, methane seep in Santa Barbara Channel<sup>10,23</sup> and methanerich ODP Hole 680B off Peru<sup>8</sup>. These taxa include species of *Bolivina*, *Cassidulina*, *Chilostomella*, *Cibicides*,

Earlier studies have observed benthic foraminifera in

methane production and gas hydrate precipitation.

Figure 3. Relative abundance of some benthic foraminifera from gas hydrate-rich Hole 997A, Blake Nose area. Methane-loving benthic foraminifera *Bolivina paula*, *Cibicides kullenbergi*, *Uvigerina hispidocostata* and *Uvigerina peregrina* are more abundant with large fluctuations in the gas hydrate-rich interval (light grey shaded bar). *Pyrgo lucernula*, a well-oxygenated species shows abundance beyond the gas hydrate interval. Extension of hydrate-charged sediments is shown by dark grey shaded bar.

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Figure 4. Map showing hydrate charged areas in the continental shelf of eastern and western Indian coast using BSRs<sup>43</sup>.

*Epistominella, Gavelinopsis, Globobulimina, Osangularia, Nonionella, Trifarina* and *Uvigerina*. High bacterial populations or bacterial mats in the seep zone attract populations of *Chilostomella, Nonionella* and *Uvigerina* as the source of food<sup>8,9,24</sup>. Bolivinids and Uvigerinids are common in areas with high and sustained food supply and low oxygen conditions, and are useful indicators for the detection of methane-rich environments<sup>6,8–10,13,25–27</sup>.

*B. paula* is commonly found in organic carbon-rich sediments in the Atlantic (C. W. Smart, pers. commun.). *C. kullenbergi* is reported in warm deep-waters of the Pacific and the Indian Oceans<sup>5,6,28</sup>. The genus *Pyrgo*, comprising such species as *P. lucernula* and *P. murrhyna*, is a good indicator of well-oxygenated environment<sup>29–32</sup>.

High relative abundances of B. paula, C. kullenbergi, U. hispido-costata and U. peregrina within the hydratecharged sediments of Hole 997A thus indicate presence of low oxygen and high organic carbon-rich environment during 5.44-2.95 Ma (433 to 180 mbsf). Almost complete absence of P. lucernula in the gas hydrate zone suggests that this species cannot tolerate low-oxygen stressful environment. We report B. paula in a methane-rich environment. Methane in Hole 997A is of biogenic origin as inferred on the basis of high organic carbon content<sup>14</sup>, which stimulates rich bacterial populations that serve as food for benthics<sup>33</sup>. Presence of low oxygen and high organic carbon benthic foraminifera supports the biogenic origin of gas hydrates in Hole 997A. The pronounced changes in methane-loving benthic fauna during 2.9-2.5 Ma at Hole 997A (dark grey bar, Figure 3) suggest large methane fluxes, which could be linked to leaking of methane due to gas hydrate instability by lowering of sea level during intensification of the Northern Hemisphere glaciation<sup>34–36</sup>.

Seven gas hydrate-rich sites have been inferred using BSRs along the eastern and western continental margins of India<sup>37- $\bar{4}1$ </sup> (Figure 4). The Arabian Sea and the Bay of Bengal with thick pile of sediments (3-4 km) and high organic carbon content (in the Arabian Sea, total organic carbon (TOC) ranges from 0.48 to 4% and in the Bay of Bengal from 0.26 to 2%), are potential areas for gas hydraterich zones. In 1996, the Petroleum Ministry, Government of India formed the National Gas Hydrate Programme (NGHP) to evolve a core group for the investigation and production of gas hydrates from the Indian continental shelf (within our Exclusive Economic Zone) in collaboration with the Gas Authority of India Limited, Oil and Natural Gas Corporation, Directorate General of Hydrocarbons, National Geophysical Research Institute and National Institute of Oceanography. Initial studies from the east coast (water depth 850 m), Andaman offshore (water depth 1400 m) and Krishna-Godavari offshore (water depth 1300 m) indicate presence of BSRs in these areas<sup>42</sup>. Most of the BSRs in the Arabian Sea are concentrated on the northern fringe of the Laccadive Ridge. Different offshore parameters favourable for the formation of gas hydrate are present in the sediments of the Indian continental margin. Suitable water depth, high organic carbon content, huge sediment thickness, high sedimentation rates and bottom water temperatures are all ideal for the formation of gas hydrates. The amount of estimated gas hydrates (trillion cubic metre, TCM) based on seismic analogue studies in different areas are (with >95% probability): Bombay offshore, 135 TCM; Kerala-Konkan offshore, 62 TCM; Northern Arabian Sea play, 226 TCM; Southern Arabian Sea Play, not known; Eastern offshore, 1038 TCM; Northern Bay of Bengal play, 245 TCM and Southern

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Bay of Bengal play, 188 TCM. Total estimated reserves of gas hydrate in these regions might be 1894–14572 TCM (NGHP estimate)<sup>43</sup>. Study of benthic foraminifera, TOC and carbon isotope values of benthic foraminifera from these sediments will give a good estimate about the actual expansion of the hydrate-rich sediments, along with release of methane during the late Neogene.

Benthic foraminiferal study from the Blake Ridge indicates that highly stressful methane-rich zone is favoured by the species characteristic of low oxygen and high organic carbon-rich environment and is not suitable for well-oxygenated species like *P. lucernula*. Our results indicate the presence of gas hydrate zone at Hole 997A extending up to 2.5 Ma.

Hydrate-charged sediments inferred by BSRs along the eastern and western continental margins of India, show similar pattern as the Blake Ridge deposits and are ideal for foraminiferal study, which can provide an insight into the extent of the gas-charged sediments and genesis of methane. With depleting natural resources, gas hydrates will continue to be an alternative future energy resource. There are vast unexplored gas-hydrate reservoirs trapped in marine sediments, which require multifaceted extensive efforts to bring them into the world energy balance. Though economic exploration of gas hydrates still remains a problem, with developing technologies in future, it could prove to be good resource of energy requirements in the world. Since India has limited petroleum reserves, with increased energy demands, efforts should be intensified to look for alternative energy sources and to use her large human resources in the exploration of gas hydrates along the vast continental margin. The country has a large coast, which serves as a depocentre for the accumulation of sediments rich in organic carbon, suitable for the formation of gas hydrates.

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# Frequencies of *CETP* gene *TaqI* B and D442G polymorphisms in North Indian population

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In this study we have analysed the frequencies of TaqI B and D442G polymorphisms of CETP gene and lipid profile in 315 healthy individuals from North Indian population. For genotyping, PCR was followed by restriction digestion with TaqI enzyme. Frequencies of B1B1: B1B2: B2B2 genotypes were 25.4%: 54%: 20.6% for TaqI B polymorphism. Our study showed that B1B1 genotype frequency is lower and B1B2 frequency is higher than other populations. D442G polymorphism was absent in North Indian population. In spite of high frequency of B2 allele, the HDL-cholesterol levels were low. In conclusion, CETP TaqI B polymorphism shows distinct pattern of genotype and allele frequency in North Indians and D442G mutation is absent. However, CETP TaqI B polymorphism was not found to be associated with circulating lipid levels.

VARIATIONS in total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol and triglyceride have been associated with increased risk of various diseases or complications associated with diseases like atherosclerosis and diabetes mellitus<sup>1</sup>. During the past 30 years, the rates of coronary artery disease (CAD) have doubled with privileged circumstances and urbanization in rural villages in India. Genetic preponderance may also alter risk for CAD by influencing lipid profile. Establishment of genetic risk factors may help in recognition of the population at risk and ultimately the management of disease.

Cholesterol ester transport protein (CETP) mediates the exchange of lipids between antiatherogenic HDL and atherogenic apolipoprotein B containing lipoproteins; thereby it potentially regulates steady-state levels of HDL-cholesterol as well as LDL-cholesterol. CETP can be antiatherogenic because of its roles in the removal of excess cholesterol from the body via LDL receptor-mediated uptake in the liver and excretion in the bile. On the other hand, it may lower the concentration of atheroprotective HDL-cholesterol<sup>2,3</sup>.

The *CETP* gene encompasses 16 exons and has been localized on chromosome 16q21. Several polymorphisms of the *CETP* gene have been reported, which may be associated with alteration in CETP activity. *TaqI* B polymorphism is most widely studied, which is created by a silent mutation in nucleotide 277 in intron 1, resulting in two alleles B1 and B2. Allele B2 with low CETP mass and increased

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