

Buried in time: Culturable Fungi in a Deep-sea Sediment Core from the Chagos Trench, Indian Ocean

Chandralata Raghukumar^{1*}, Seshagiri Raghukumar¹, G. Sheelu², S.M. Gupta¹,
B. Nagender Nath¹, B.R. Rao¹

¹ National Institute of Oceanography, Dona Paula, Goa – 403 004, India, and

² Indian Institute of Chemical Technology, Uppal Road, Hyderabad – 500 007, India.

Abstract

The recovery of culturable microorganisms from ancient materials, ranging from a few thousand to several million years old, has generated interest in the long-term survival capabilities of microorganisms. We report the occurrence of such paleobes for the first time from a deep-sea sediment core obtained from a depth of 5904 m from the Chagos trench in the Indian Ocean. Culturable fungi, direct counts of bacteria, age of the sediments based on the radiolarian assemblage, total organic carbon, Eh and CaCO₃ were determined in these sediments. Culturable fungi were obtained from subsections up to 370 cm depth. The age of the sediments from which fungi were isolated was estimated to range from > 0.18 to 0.43 Ma (Million years), being the oldest recorded age for recovery of culturable fungi. Colony forming units of fungi ranged from 69 to 2493 g⁻¹ dry weight sediment with a maximum abundance recorded at 160 cm depth of the core, corresponding to ~ 0.18 Ma. Bacterial numbers in the core showed oscillations corresponding to cycles of approximately 100 Ka (kilo years). The fungi comprised non-sporulating forms and a sporulating form identified to be *Aspergillus sydowii*. Germination of spores of *A. sydowii* at 100, 300 and 500 bar hydrostatic pressures at 5°C confirmed its barotolerance and its nativity to deep-sea sediments. We propose that deep-sea sediments are a source of paleobes, which could be useful in studies on palaeoclimate, long-term microbial survival and biotechnology.

Keywords: Central Indian Ocean; Chagos Trench; Sediment core; Ancient fungi; Palaeoclimate

*Corresponding author. Fax: (91) 832-2450602;
E-mail address: lata@darya.nio.org (Chandralata Raghukumar)

1. Introduction

Deep-sea surface sediments harbour bacteria and fungi that sink from the overlying waters (Takami et al., 1997). It is likely that due to a further deposition of sediments such microorganisms gradually get buried in deeper layers. Do cells and spores of such microorganisms remain viable, and if so, for how long? Viable microorganisms, preserved alive for thousands to millions of years have been reported from several terrestrial sources of ancient origin. Some of these are 25 to 40 million year (Ma) old extinct bees buried in amber (Cano and Borucki, 1995), intestinal contents of 12,000 year old mastodon remains (Rhodes et al., 1998), 400,000 year old deep ice cores in the Antarctic ice sheet (Abyzov, 1993; Castello et al., 1999), subsamples from a nearly 2000 m long glacial ice core (Ma et al., 2000) and 50 m deep core samples from 3 million years old Siberian permafrost (Shi et al., 1997). Evidence also exists for the presence of viable microbes in habitats that have remained isolated from contact with others for a long time. Thus, viable, and possibly actively growing microorganisms are suspected to inhabit Lake Vostok, Antarctica, isolated by about 4000 m in depth from overlying ice and for nearly 1 million years in time (Karl et al., 1999; Priscu et al., 1999). Terrestrial deep mud cores and aquifers are another habitat for such ancient microorganisms (Balkwill, 1989; Balkwill et al., 1989; Beeman and Sulfito, 1990). Studies on such culturable ancient microorganisms are extremely important in understanding long term biological survival mechanisms, as well as in palaeoclimatology (Abyzov, 1993).

Few studies have considered deep-sea sediment cores as a source of ancient organisms. An exception is the study on thermophilic bacteria in 5,800 year old sediments from an ocean basin (Bartholomew and Paik, 1966). Nealson and Inagaki (2003) have used the term "paleobes" for the collection of rDNA sequences of microorganisms in ancient relics, a term also used in this paper for microbes recovered from ancient material. While most studies have focussed on bacteria, reports on fungal are rare (Ma et al., 2000). We had an opportunity to study these organisms during participation in a cruise on board the research vessel AA Sidorenko to the Central Indian Ocean area to investigate the deep-sea benthic environment. In this paper, we

report the occurrence of culturable bacteria and fungi in ~0.18 to 0.43 million years (Ma) old subsurface deep-sea sediments, and further propose that these are a repository of paleobes.

2. Methods

2.1. Study site

The sediment core was taken at a depth of 5904 m from a trench at the southern extension in the Chagos-Laccadive ridge system (11°06'S, 72°31'E) in the Indian Ocean (Fig. 1). This trench is among the deepest regions of the Indian Ocean. Like other trench systems, it is oriented parallel to a volcanic arc.

2.2. Sampling

The sediment core measuring 460 cm was collected using a gravity core of 10 cm diameter PVC pipe. Possible contamination of ancient samples by extant organisms is a major issue in the study of paleobes. Therefore, stringent measures against contamination were maintained throughout the sample collection and microbial isolation. As soon as the core was brought on board, samples were collected by inserting a sterile plastic bag at the lower end of the core, into which 5 cm long sediment samples were extruded. This helped to avoid aerial contamination. The bags were closed with rubber bands and transported to the laminar flow hood in the laboratory on board, where a portion of the sediment from the middle of each sub sample that had not been in contact with the walls of the core pipe was removed using an alcohol-flame sterilized spatula and placed in sterile containers for isolation of bacteria and fungi.

2.3. Culturing of bacteria and fungi

Fungi were cultured by surface plating from sediments on board the research vessel. Dilute culture media were used in order to provide low nutrient conditions. Approximately 1 g of sediment was suspended in 10 ml of sterile sea water and vortexed for 1 min. Three replicates of 0.1 ml sediment suspension were spread on five fold diluted Malt Extract Agar (MEA) medium (HiMedia, India) prepared with sea water

and fortified with streptomycin (0.1 g in 100 ml medium) and penicillin (10,000 Units in 100 ml medium) to inhibit bacterial growth. In addition, we also isolated bacteria by spread-plating replicate samples on nutrient agar medium diluted 5 times and prepared with seawater. The plates were incubated at 5-7°C. Nutrient plates exposed to air for 10 minutes on the deck of the research vessel where the core was received, the microbiology laboratory on board and the laminar flow inoculation hood served as control plates. These did not yield any fungal colonies, thus ruling out aerial fungal contaminations.

2.4. Dating of the sediments using biostratigraphy

Age zonation within the sediment sections was determined using radiolarian biostratigraphy based on the first appearance datum (FAD) and last appearance datum (LAD) of the radiolarian index species (Johnson et al., 1989). Sediment aliquots of ~5 ml were dispersed in water, treated with hydrogen peroxide (30%) and sieved through >62 µm mesh. The coarse fraction was mounted in Canada balsam for making permanent slides (Johnson et al., 1989). The slides were scanned at 100 x magnification under a Laborlux-2 (Leitz, Germany) microscope with an image analysis system. The stratigraphically important pylonids, the surface water dwelling (0-50m) radiolarian species for the Late Quaternary, were searched and identified. These are very sensitive to the changes in the sea surface temperature (SST), which is governed by the incoming solar radiation resulting from the variations in the Earth's orbital eccentricity (e), axial tilt (t) and the precession of equinoxes (p). In brief, in the geological past the pylonids responded to the Earth's orbital forcing, which is defined as the cumulative and normalized sum of the eccentricity, tilt and precession, the ETP (Gupta, 1999, 2002). The ETP forcing contains the 100 thousand kilo years (ka) orbital-eccentricity, 41 ka axial-tilt, and 23 ka precession cycles, and if any of these climatic cycles match the proxy-climate (herein the pylonids) signals, the paleoclimatic results are considered to be of very high significance. We used pylonids abundance and the ETP's 100 ka eccentricity related curve to interpret our data in the present study.

2.4. Direct detection of fungi in the sediments

One ml of filter-sterilized solution of the optical brightener, Calcofluor (Sigma Chemicals, USA), at a concentration of 0.1 % w/v was added to about 0.1 g of sediment (Mueller and Sengbusch, 1983). A drop of sterile detergent solution was added to this and vortexed vigorously in order to separate lighter detritus and fungal structures from the heavier mineral portion of the sediment. The resulting foam was examined under an epifluorescence microscope (Olympus BX 60, Japan) for fungal hyphae and conidia.

2.5. Total bacterial counts

A small amount of the sediment (~ 1.0 g) was taken in 9 ml of filtered, sterile sea water and vortexed for 1 min. A volume of 3 ml from this was then fixed with phosphate buffered formalin (5 % final concentration) and a 900 μ l aliquot was vortexed for 1 min. This sample was filtered over black polycarbonate filters of 0.22 μ m pore size and stained with 0.01 % aqueous solution of acridine orange for 3 min. Bacterial cells were counted from 10 – 20 microscopic fields, using an epifluorescence microscope (Olympus BX 60). The final numbers are expressed as total counts g^{-1} dry sediment (Parsons et al., 1984).

2.6. Total organic carbon (TOC) and Eh

TOC was measured by wet oxidation method followed by titration with ammonium ferrous sulfate and expressed as percentage (El Wakeel and Riley, 1956). Total organic matter (TOM) was derived from TOC using a conversion factor of 1.82. A precision of 0.01% C is routinely obtained in our laboratory. Eh was analysed immediately after retrieving the core on board the research vessel using a combination of glass Eh electrodes mounted on a stand. The electrode was slowly wound down into the sediment (Pearson and Stanley, 1979) and allowed to stay for a minimum period of 60 seconds. Between each set of readings, electrodes were rinsed with distilled water and then standardized against ferrocyanide-ferricyanide redox buffer. Readings were made on digital mV/pH meter capable of reading to 1 mV difference and were corrected for hydrogen references by adding +198 to the direct reading obtained on the mV meter.

2.7. Dry bulk density of *sediments*

Dry bulk density (DBD) was empirically calculated from sediment CaCO₃ data using the non-linear equation ($=3.104 \times 10^{-5}(\% \text{CaCO}_3)^2 + 2.176 \times 10^{-3}(\% \text{CaCO}_3) + 0.430$) as described by Clemens et al. (1987). Calcium carbonate content of sediments was determined by the “Karbonat-Bombe” method (Müller and Gastner, 1971). The DBD is one among the several physical properties of sediment including porosity and is inversely related to porosity as shown by the equation of Garg (1987).

2.8. Growth under hydrostatic pressure

An isolate of *Aspergillus sydowii* obtained in culture from the sediment core at 160 cm depth was grown on Czapek-Dox Agar medium. Spores from a growing culture were harvested and a dilution of spore suspension in sterile sea water was prepared and spread on sea water agar medium. Discs of 5 mm diameter containing the spores were cut from the agar plates, introduced into sterile gas permeable polypropylene sterile pouches that were filled with sterile seawater and sealed without trapping any air bubbles. These were suspended in a deep-sea culture vessel (Tsurumi & Seiki Co., Japan) filled with sterile distilled water and pressurized to the desired hydrostatic pressure and incubated at 5° C. Three replicates were maintained for each treatment. After 10 days of incubation, the deep-sea culture vessels were decompressed gradually and the contents of the pouches were immersed in lactophenol-cotton blue stain. Percentage germination of spores was estimated from 25-30 microscopic fields, each containing 15-20 spores (Raghukumar and Raghukumar, 1998).

3. Results

Biostratigraphic dating using radiolarian fossil index species showed three distinct litho-biounits of the sediment core (Table 1). The core section between 20 and 160 cm was characterized by the presence of two extant radiolarian species *Buccinosphaera invaginata* and *Collosphaera tuberosa*, which had first appearance datum (FAD) of 0.18 and 0.65 Ma, respectively. *B. invaginata* was not found below 280 cm. Therefore, this unit of the core up to 280 cm, was estimated to be younger than

0.18 Ma. *Collospira tuberosa* was found to occur up to a depth of 370 cm, while the fossil species *Stylatractus universus*, with a last appearance datum (LAD) of 0.43 Ma, was detected below this depth. The second unit between 280 and 370 cm, was, therefore, estimated to be >0.18 to < 0.43 Ma. The upper series at 0 – 370 cm of the core was separated from the older series at a depth of 370 cm by a distinct geological break (hiatus?), a period of non-deposition or erosion. This series below 370 cm was characterised by the presence of the very old index fossils like *Pterocanium prismatium* (LAD of 1.6 Ma), *S. universus* (LAD of 0.43 Ma), *Anthocyrtidium angulare* (LAD of 1.0 Ma) and *Theocorythium vetulum* (LAD 1.6 Ma). The lower series below 370 cm can, therefore, be dated as being older than 0.43 Ma (Table 1).

We successfully cultured fungi from 6 out of 22 subsections of a core between 0 and 460 cm. A distinct stratification in the numbers of fungi was detected in the core (Table 1). No fungi were recovered at depths of 0 to 10 cm, while samples from 15 to 50 cm yielded 69 to 446 fungal colony forming units (CFU) g⁻¹ dry weight sediment. All the recovered fungi between 15 and 50 cm were non-sporulating. Relatively high densities of sporulating colonies (2493 CFU g⁻¹ of dry sediment weight) were recovered at a depth of 160 cm, corresponding to ca. 0.18 Ma age. This sample yielded almost exclusively the mitosporic fungus *Aspergillus sydowii* (Bain & Sart) Thom & Church. (The identification was further confirmed by Prof. Walter Gams at the Central bureau voor Schimmelcultures, Baarn, Netherlands). Considerable densities of this fungus, as well as non-sporulating forms were also present at depths of 280 and 370 cm, with an age between 0.18 and 0.43 Ma (Table 1). The older series below 370 cm (>0.43 to 1.6 Ma), did not reveal the presence of culturable fungi. Culturable bacterial numbers varied from 10 to 2030 CFU g⁻¹ sediment, with a maximum density at 10 cm (Table 1).

We confirmed the presence of fungi in the sediments from various subsections of the core through direct microscopic detection of fungal hyphae and spores by staining with the optical brightener Calcofluor (Fig. 2a, b). Only sediment particles, but not the fungal filaments were detectable using bright field microscopy. Using the epifluorescence field fungi could be visualized (Fig. 2b). The relation between sediment

particles and fungal hyphae could be clearly made out using both epifluorescence and bright field together (Fig. 2a).

Spores from the culture of *A. sydowii* germinated and grew at elevated hydrostatic pressures of 100, 300 and 500 bars, corresponding to 1000, 3000 and 5000 m depths, and cold temperatures of 5°C (Table 2). The morphology of the fungus at ambient atmospheric pressure and temperature was abnormal during the initial isolation, showing unusually thick hyphae with swellings, long conidiophores, imperfectly formed conidiogenous cells and poor sporulation.

Dry bulk density derived from %CaCO₃, TOM content, Eh profiles show distinct downcore distributional patterns indicating the preservation of stratification necessary for palaeoclimate studies (Figs. 3c-d).

4. Discussion

We isolated numerous fungi from the core samples, most of which were non-sporulating and, therefore, not identifiable. However, some of the cultures sporulated and could be identified as belonging to *Aspergillus sydowii*. This species, which was present at core depths of 160 to 370 cm, is a common terrestrial fungus and has been reported from a wide variety of habitats, including deglaciated soils in Alaska (Domsch et al., 1990). Dry spores of terrestrial, mitosporic fungi, such as *A. sydowii* can be easily transported by wind during dry periods (Dix and Webster, 1995) and can reach fresh habitats, such as the marine. It has recently been suggested that spores of *A. sydowii* blown from the Saharan deserts during dust storms, reach the Caribbean islands in the Pacific and cause the aspergillosis disease in seafans (Shinn et al., 2000; Smith et al., 1996). It is likely that such spores might eventually sink to the deep-sea surficial sediments, undergo natural selection mechanisms with time and acquire capabilities to grow and multiply in the presence of suitable nutrient sources. Spores of our isolate of *A. sydowii* from the core samples germinated and grew at elevated hydrostatic pressures and low temperature (Table 2). In addition, the fungus was morphologically abnormal during the early periods of subculture. The studies of Geiser et al. (1998) and Alker et al. (2001), have shown that the aspergillosis disease of

seafans is caused by physiologically distinct marine strain of the terrestrial fungus *A. sydowii*. Unadapted terrestrial strains of fungi are unlikely to grow under the elevated hydrostatic pressures found in the deep sea (Raghukumar et al., 1992; Lorenz, 1993).

Fungi in deep-sea surface sediments might eventually be buried in time, with deposition of sediments above. The sedimentation rates in the Central Indian Ocean are reported to be ~1 cm per 1000 years (Gupta, 1999). Therefore, we believe that the fungi that we recovered from the core were buried during a certain period of time before the recent past. Likewise, several terrestrial fungal species have also been recovered from polar glacial ice, apparently the spores having arrived from around the world and being entrapped in ice (Ma et al., 2000). Direct DNA amplifications from ice subcore melt-water further confirmed observations of the presence of terrestrial species of fungi (Ma et al., 2000). Several facts suggest that the fungi that we recovered from the cores were deposited in ancient times and had not seeped from recent sediments on the surface. Thus, *A. sydowii* occurred in dense numbers only at one section of the core. Fungi were not present in the upper 10 cm core samples, corresponding to recent sedimentation. The localized occurrence of fungi that we found is not to be expected if seepage from surface had occurred. Further, the maximum number of fungal colonies that we found were recovered at 160 cm depth, which is capped off by a less porous sediment having higher dry bulk density at 70 cm depth than at other depths (Fig. 3d). A lack of distinct porous layers (Fig. 3d) including the absence of porous rock pieces such as pumice stones was observed in these sediments.

Our observations have implications on the long time survival of microorganisms, which has fascinated many researchers. The oldest of these are believed to be 25 to 40 Ma old (Cano and Borucki, 1995). One of the few studies on fungi has shown the survival of fungal spores for a period of up to 0.14 Ma in polar glacial ice (Ma et al., 2000). The present study shows for the first time that fungi lay protected for at least 0.43 Ma at 160 - 370 cm in the deep-sea sediment core obtained from a depth of 5904 m from the Chagos Trench in the Indian Ocean. To our knowledge, these are the most ancient fungi recovered in culture so far. Long-term survival of microorganisms is affected by the limited chemical stability of DNA, which undergoes significant rates of

hydrolysis, oxidation and non-enzymatic methylation *in vivo* (Lindahl, 1993). Low temperature conditions will favour mitigation of DNA damage thus enabling isolation of viable organisms from deep ice cores. Likewise, elevated hydrostatic pressure, as in the deep-sea, is another possible mechanism for preservation (Colby et al., 1993). It is likely that a combination of both will be extremely favourable for long-term preservation.

The distinct stratification of fungal numbers and species in the sediment core, accompanied by the various other characteristics suggest that deposition of fungi and other particles on bottom sediments from overlying waters, as well as active growth and multiplication of these organisms is not uniform in time, probably depending upon the prevailing climate. Since fungal spores are transported during dry periods, we postulate that fungal paleobes in the ancient material of deep-sea core sediments might provide a clue to the prevailing atmospheric circulation, winds and climate. The late Pleistocene, to which the present sediment samples belonged had witnessed warm and cold climates at cyclicities of 0.1 Ma (or 100 ka) due to the earth's eccentricity, axial tilt and precession cycles (Imbrie and Imbrie, 1986; Gupta, 2002; Gupta, 2003) (Fig. 3a). Three significantly warm periods existed between 0.18 and 0.43 Ma (Fig. 3a). It was from this sub sample that *A. sydowii* was isolated.

While culturable numbers of bacteria showed no distinct stratification, acridine orange direct counts revealed regular oscillations at intervals of approximately every 50 cm core depth (Fig. 3b). The stratification in total bacterial numbers, total organic matter (TOM) and Eh suggest a relationship between paleobes and geochemical properties of the sediments. Elevated levels of TOM corresponded to low Eh at about 250 cm with high total bacterial numbers (Fig. 3c). Organic carbon in sediments (along with other proxies) is a good proxy for palaeoproductivity (Lyle, 1988). Higher bacterial abundance at these depths may probably suggest that the geological sections showing higher productivity may also be reflected by an increase in bacterial abundance. It might be worthwhile to further examine the relation between the 100 kilo year cycles of earth's orbital eccentricity, axial tilt and precession (K ETP) and cycles of warm and cold periods (Fig. 3a) as inferred from the pyloniid group of radiolarians (Gupta, 2003).

In conclusion, we report the occurrence of paleobioic fungi in deep sea cores with an estimated range in age of > 0.18 to 0.43 Ma (Million years), the oldest recorded age for recovery of culturable fungi. The recovered fungi might have been derived from past hyphae or spores that had been transported from land, buried, and preserved for long periods of time. Our study extends the presence of paleobioes to deep sea cores, besides polar ice cores and terrestrial subsurface sediments. Since ancient viable fungi in deep-sea sediment cores can be cultured and identified, these could be valuable in palaeoclimate studies. Such fungi also provide an opportunity to understand cellular adaptations involved in long-term survival. Future studies on paleobioes will allow us a virtual time travel to the past and pick up organisms for potential biotechnological explorations.

Acknowledgments

We thank the Captain and crew of the Russian research vessel A.A. Sidorenko for co-operation. Our sincere thanks to our former Director, Dr. Ehrlich Desa for critical improvement of the manuscript. The oceanographic cruise to the Indian Ocean was financed by the Department of Ocean Development, New Delhi. This is NIO's contribution No. 3908.

References

- Alker, A.P., Smith, G.W., Kim, K., 2001. Characterization of *Aspergillus sydowii* (Thom et Church), a fungal pathogen of Caribbean seafan corals. *Hydrobiologia* 460, 105-111.
- Abyzov, S. S., 1993. Microorganisms in the Antarctic ice. In: Friedman, E.I. (Ed.), *Antarctic Microbiology*. Wiley-Liss, New York, pp. 265-295.
- Balkwill, D. L., 1989. Numbers, diversity and morphological characteristics of aerobic, chemoheterotrophic bacteria in deep subsurface sediments from a site in South Carolina. *Geomicrobiology Journal* 7, 33-52.
- Balkwill, D. L., Fredrickson, J. K., Thomas, J. M., 1989. Vertical and horizontal variations in the physiological diversity of the aerobic chemoheterotrophic bacterial microflora in deep southeast coastal plain subsurface sediments. *Applied and Environmental Microbiology* 55, 1058-1065.
- Bartholomew, J. W., Paik, G., 1966. Isolation and identification of obligate thermophilic spore forming bacilli from ocean basin cores. *Journal of Bacteriology* 92, 635-638.
- Beeman, R.E., Sulfito, J.M. 1990. Evaluation of deep subsurface sampling procedures using serendipitous microbial contaminants as tracer organisms. *Geomicrobiology Journal* 7, 223-233.
- Cano, R. J., Borucki, M.K., 1995. Revival and identification of bacterial spores in 25-to 40-million-year-old Dominican amber. *Science* 268, 1060-1064.
- Castello, J. D., Rogers, S.O., Starmer, W.T., Catranis, C., Ma, L., Bachand, G., Zhao, Y., Smith, J.E., 1999. Detection of tomato mosaic virus RNA in ancient glacial ice. *Polar Biology* 22, 207-212.
- Clemens, S.C., Warren, L.P., Howard, W.R., 1987. Retrospective dry bulk density estimates from southeast Indian Ocean sediments-Comparison of water loss and chloride-ion methods. *Marine Geology* 76, 57-69.

Colby, J. W., Enrriquez-Ibarra, L. G., Flick, G. J., 1993. The shelf life of fish and shell fish. In: Charalambous, G. (Ed), Shelf life studies of foods and beverages. Elsevier, New York, pp. 85-144.

Dix, N.J., Webster, J., 1995. Fungal Ecology. Chapman and Hall, London.

Domsch, K. H., Gams, W., Anderson, T-H., 1980. Compendium of soil fungi. Academic Press, London.

El Wakeel, S. K., Riley, J.P., 1956. The determination of organic carbon in marine muds. Conseil International Pour l'exploration de la mer 22, 180-183.

Garg, S.K., 1987. Soil Mechanics. Khanna Publ., New Delhi.

Geiser, D.M., Taylor J.W., Kim, B.R., Smith, G.W., 1998. Cause of seafan death in the West Indies. Nature 394,137-138.

Gupta, S.M., 1999. Radiolarian monsoonal index: Pyloniid group responds to astronomical forcing in the last~500,000 years:Evidence from the Central Indian Ocean. Man and Environment 24, 99-107.

Gupta, S. M., 2002. Pyloniid stratigraphy- a new tool to date tropical radiolarian ooze from the central tropical Indian Ocean. Marine Geology 184, 85-93.

Gupta, S. M., 2003. Orbital frequencies in radiolarian assemblages of the Central Indian Ocean; implications on the Indian summer monsoon. Palaeogeography, Palaeoecology, Paleoclimatology 197, 97-112.

Imbrie, J., Imbrie, K.P., 1986. Ice Ages: Solving the mystery. Harvard University Press, Cambridge.

Johnson, D. A., Schneider, D.A., Nagrini, C.A., Caulet, J.P., Kent, D.V., 1989. Pliocene-Pleistocene radiolarian events and magneto-stratigraphic calibrations for the tropical Indian Ocean. Marine Micropalaeontology 14, 33-66 .

- Karl, D.M., Bird, D.F., Björkman, K., Houlihan, T., Shackelford, T., Tupas, L., 1999. Microorganisms in the accreted ice of Lake Vostok, Antarctica. *Science* 286, 2144-2147.
- Lindahl, T., 1993. Instability and decay of the primary structure of DNA. *Nature* 363, 709-715.
- Lorenz, R., 1993. Kultivierung mariner Pilze unter erhöhtem hydrostatischen Druck. Ph.D thesis. University of Regensburg.
- Lyle, M. 1988. Climatically forced organic carbon burial in the equatorial Atlantic and Pacific Oceans. *Nature* 355, 529-532.
- Ma, L., Rogers, S.O., Catranis, C., Starmer, W.T., 2000. Detection and characterization of ancient fungi entrapped in glacial ice. *Mycologia* 92, 286-295.
- Müller, G., Gastner, M., 1971. "The Karbonat-Bombe", a simple device for the determination of carbonate content in sediments, soils and other material. *Neues Jahrbuch Mineralogie*, 10, 466-469.
- Mueller, V., Sengbusch, P. V., 1983. Visualization of aquatic fungi (Chytridiales) parasitising on algae by means of induced fluorescence. *Archiv fuer Hydrobiologia* 97, 471-485.
- Nealson, K.H., Inagaki, F., 2003. Endolithic bacterial RDNA components associated with the mid-cretaceous oceanic anoxic event (OAE). *Geophysical Research Abstracts* 5, 13653.
- Parsons, T. R., Maita, Y., Lalli, C.H., 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford.
- Pearson, T.H., Stanley, S.U., 1979. Comparative measurement of redox potential of marine sediments as a rapid means of assessing the effect of organic pollution. *Marine Biology* 53, 371-379.

Priscu, J. C., Adams, E.E., Lyons, W.B., Voytekogk, M.A., Brown, R.L., McKay, C.P., Takacs, C.D., Welch, K.A., Wolf, C.F., Kirshtein, J.D., Avci, R., 1999. Geomicrobiology of subglacial ice above Lake Vostok, Antarctica. *Science* 286, 2141-2144.

Raghukumar, C., Raghukumar, S., Sharma, S., Chandramohan, D., 1992. Endolithic fungi from deep-sea calcareous substrata: isolation and laboratory studies. In: Desai, B.N. (ed) *Oceanography of the Indian Ocean*. Oxford IBH Publ., New Delhi, pp. 3-9.

Raghukumar, C., Raghukumar, S., 1998. Barotolerance of fungi isolated from deep-sea sediments of the Indian Ocean. *Aquatic Microbial Ecology* 15, 153-163.

Rhodes, A. N., Urbance, J.W., Youga, H., Corlew-Newman, H., Reddy, C.A., Klug, M.J., Tiedje, J.M., Fisher, D.C., 1998. Identification of bacterial isolates obtained from intestinal contents associated with 12,000-year-old mastodon remains. *Applied and Environmental Microbiology* 64, 651-658.

Shi, T., Reeves, R.H., Gilichinsky, D.A., Friedman, E.I., 1997. Characterization of viable bacteria from Siberia permafrost by 16S rDNA sequencing. *Microbial Ecology* 33, 169-179.

Shinn, E. A., Smith, G.A., Prospero, J.M., Betzer, P., Hayes, M.L., Garrison, V., Barber, R.T., 2000. African dust and the demise of Caribbean coral reefs. *Geophysical Research Letters* 27, 3029-3032.

Smith, G. W., Ives, L.D., Nagelkerken, I.A., Ritchie, K.B., 1996. Caribbean sea-fan mortalities. *Nature* 383, 487.

Takami, H., Inoue, A., Fuji, F., Horikoshi, K., 1997. Microbial flora in the deepest sea mud of the Mariana Trench. *FEMS Microbiology Letters* 152, 279-285.

Table 1. Stratification of fungi and radiolarians in the deep-sea sediment core.

Depth (cm)	Fungal species	Fungal CFU*	Bacterial CFU*	Radiolarian index	fossils, First Appearance Datum (FAD) and Last Appearance Datum (LAD)
10	-	-	2030		
15	Unidentified non-sporulating	446	-		
20	-	-	20		
40	Unidentified non-sporulating	69	-		
50	Unidentified non-sporulating	207	60		Zone (NR-1) <0.18 Ma
70	-	-	10		
100	-	-	20		
160	<i>Aspergillus sydowii</i>	2493	-		<i>B. invaginata</i> FAD ~0.18 Ma
280	<i>A. sydowii</i> and unidentified, non-sporulating form	268	30		Zone (NR-2) > 0.18 to < 0.43 Ma <i>C. tuberosa</i> FAD ~0.65 Ma
370	<i>A. sydowii</i> and unidentified, non-sporulating form	592	-		~~~~?? Hiatus~~~~ <i>S. universus</i> LAD ~0.43 Ma
400	-	-	35		
430	-	-	-		
460	-	-	-		<i>P. prismatium</i> , LAD 1.6 Ma <i>A. angulare</i> LAD 1.0 Ma <i>T. vetulum</i> , LAD 1.6 Ma

*Colony Forming Units g⁻¹ dry sediment: NR- Neogene Radiolarian Zone

Table 2. Germination of conidia of *Aspergillus sydowii* under elevated hydrostatic pressure

Treatment	% germination of conidia*	Length of the germ tubes*
5°C/1 bar	22	nt
5°C/500 bar	4	nt
28°C/100 bar	98	133 µm
28°C/200 bar	97	117 µm
28°C/500 bar	32	80 µm

Nt= not tested. * Percentage germinated conidia and length of germ tubes were estimated after incubation under various growth conditions for 2 weeks.

Legends to the Figures:

Fig. 1. Location of the sampling site, the gravity core CG-2 in the Indian Ocean.

Fig. 2. Photomicrographs of sediment from 160 cm depth of the core, stained with the optical brightener Calcofluor. (a) epifluorescence and bright field microscopy showing fungal hypha and sediment particles; (b) epifluorescence microscopy showing fungal hypha. Bar=10 μm

Fig. 3a. 100 ka cyclic components of ETP (a sum of Earth's eccentricity, axial tilt and precession) and pyloniid (a monsoon index) eccentricity bands. Peaks represent warm and humid climate and troughs represent cold and dry climate.

Fig. 3b. Total counts of bacteria using Acridine orange direct counts (AODC) method. The standard deviations were within 5% error.

Fig. 3c. Percentage total organic matter (TOM) and the redox potential measured as Eh mv (+).

Fig. 3d. The dry bulk density (DBD) of the sediment subsamples as calculated from % CaCO_3 (see Materials and Methods). Fungal colony forming units recovered in the sediments depths are shown as numbers g^{-1} dry sediment against the DBD curve.

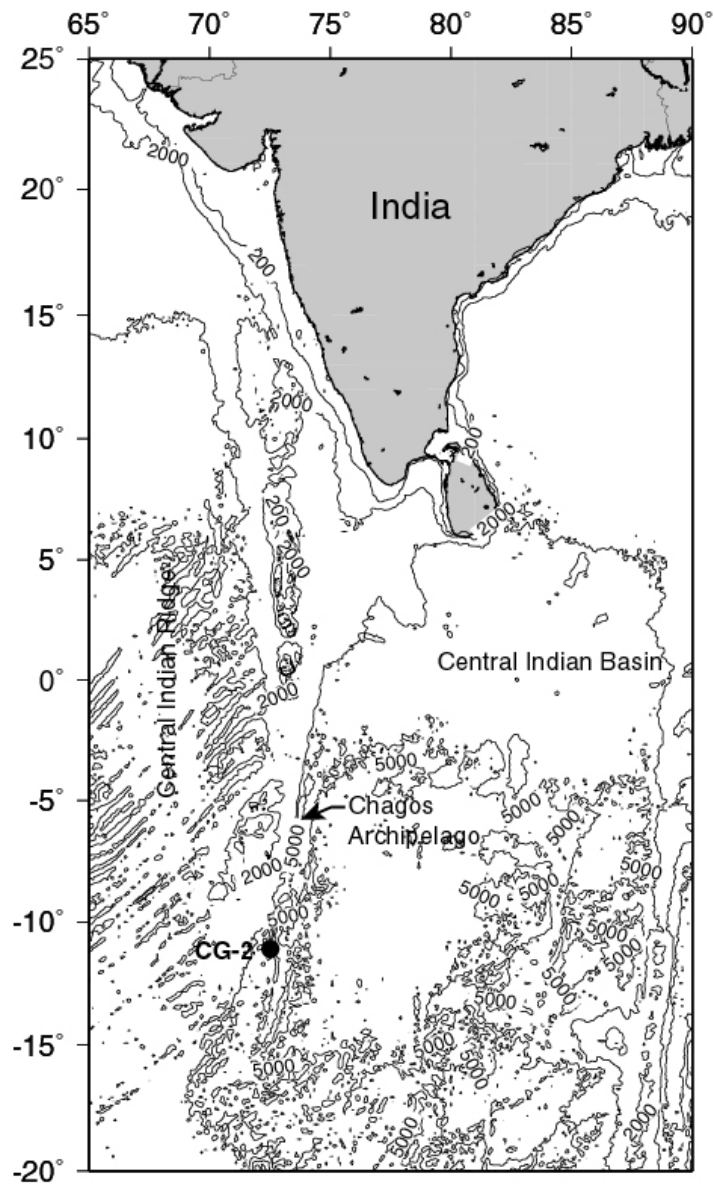


Fig. 1

Fig. 1. Location of the sampling site, the gravity core CG-2 in the Indian Ocean.

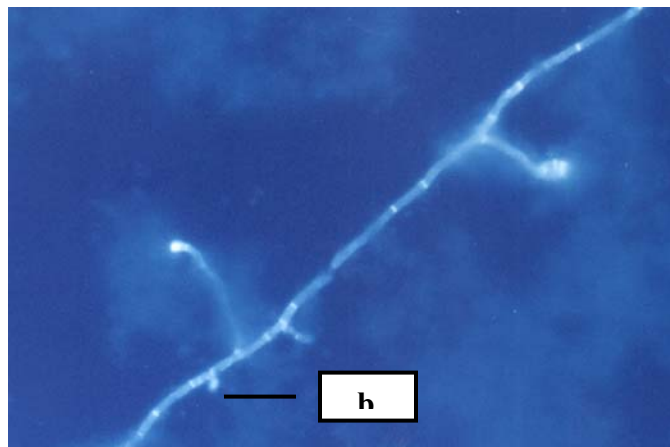
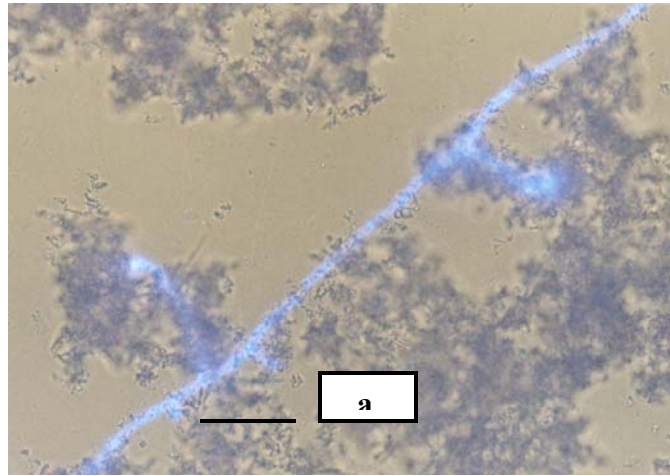


Fig. 2. Photomicrographs of sediment from 160 cm depth of the core, stained with the optical brightener Calcofluor. (a) epifluorescence and bright field microscopy showing fungal hypha and sediment particles; (b) epifluorescence microscopy showing fungal hypha. Bar=10 μ m

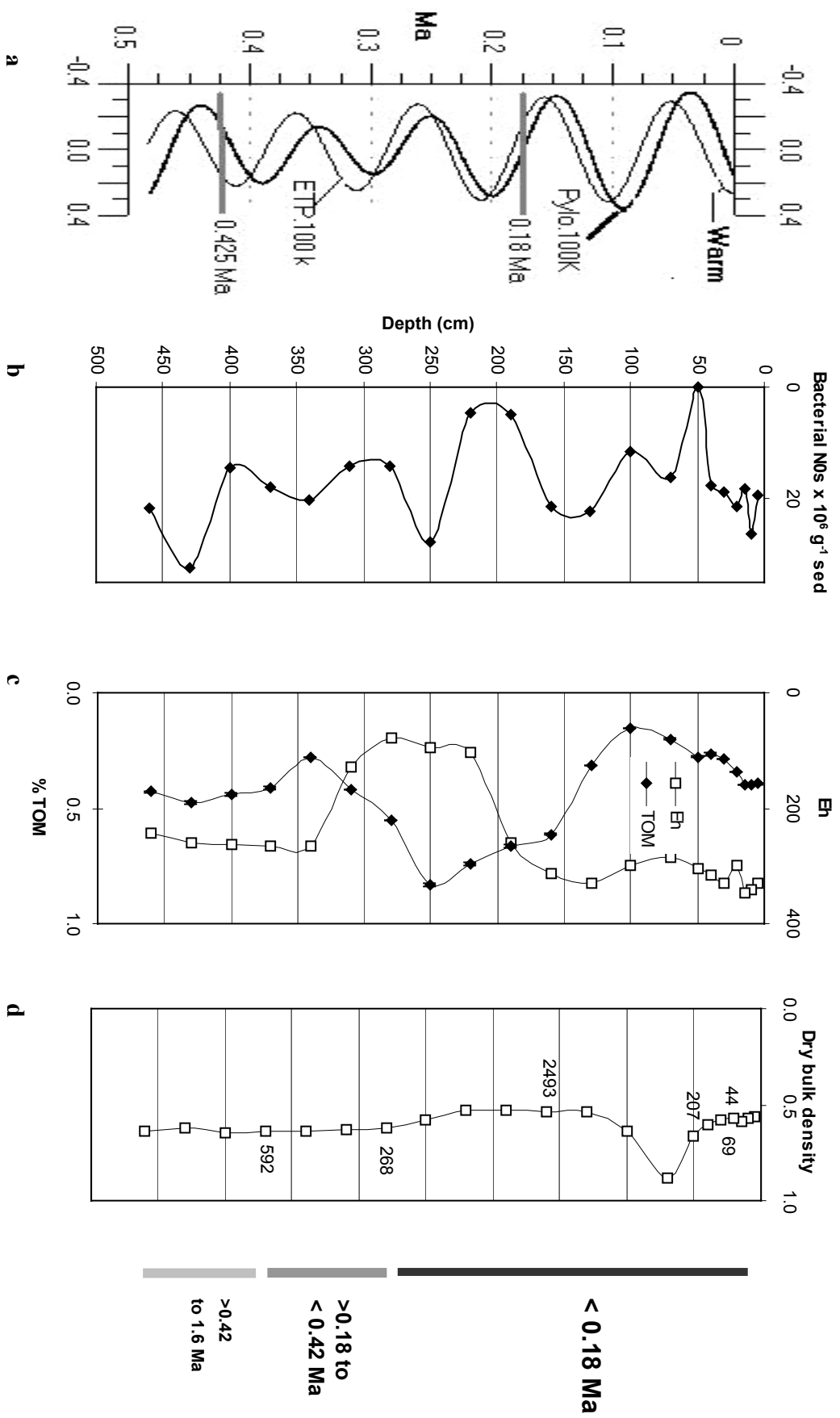


Fig. 3 (a) 100 ka cyclic components of ETP (a sum of Earth's eccentricity, axial tilt and precession) and pyloniid (a monsoon index) eccentricity bands. Peaks represent warm and humid climate and troughs represent cold and dry climate. (b) Total counts of bacteria using Acridine orange direct counts (AODC) method. The standard deviations were within 5% error. (c) Percentage total organic matter (TOM) and the redox potential measured as Eh mv (+). (d) The dry bulk density (DBD) of the sediment subsamples as calculated from % CaCO₃ (see Materials and Methods). Fungal colony forming units recovered in the sediments depths are shown as numbers g⁻¹ dry sediment against the DBD curve.