

# **Bacterial response to contrasting geochemistry in the sediments of Central Indian Ocean Basin**

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## **Abstract**

The latitudinal differences in geochemical and physiographic nature could evoke varied biological/microbiological response in the deep-sea benthic ecosystem of the Central Indian Ocean. Sediment cores have been examined along latitudinal gradient as a part of the Polymetallic Nodules-Environmental Impact Assessment- Environmental Variability Data Collection (PMN-EIA-EVDC) programme. We present here two such examples of geochemically contrasting cores namely, TVBC-26 and TVBC-08R. Core TVBC-26 (10°S, 75.5°E) is more terrigenously influenced and falls in the siliceous sediment realm and has a relatively higher total organic carbon content (0.37%). Core TVBC-08R (16°S, 75.7°E) falling in the pelagic red clay realm down south is away from the influence of the terrigenous influx and has a lesser total organic carbon value (0.22%). Though the total abundance of bacteria was one order higher in TVBC-08R ( $1.4 \times 10^9$ ) as compared to TVBC-26 ( $7.2 \times 10^8$ ), the anaerobic viability was one order high in TVBC-26. The viability of bacteria under aerobic condition was marginally high in TVBC-08R. Cells were generally  $\leq 1 \mu\text{m}$  in core 08R and ranged from 1.5-1.8  $\mu\text{m}$  in core 26. Retrievability of bacteria was in the same order ( $10^4 \cdot \text{g}^{-1}$ ) on different strengths of ZoBell Marine agar suggesting that the bacteria were nutritionally more versatile in core TVBC-08R. Besides, the retrievability of nitrifier was also one order more at this station.  $^{14}\text{C}$  uptake at 5°C for 30hrs showed that core TVBC-08R exhibited more autotrophic potential with DPM values of  $478 \pm 395 \cdot \text{g}^{-1}$  as compared to only  $17 \pm 4 \cdot \text{g}^{-1}$  in core TVBC-26. Thus, under the influence of nutrient starved North Atlantic Deep-Waters [28] the bacterial community at TVBC-08R are nutritionally more flexible as compared to that at TVBC-26, which is bathed by the nutrient-rich Antarctic Bottom Waters [9].

## Introduction

The Central Indian Ocean Basin (CIOB) has an area of  $5.7 \times 10^6$  km<sup>2</sup> and is bordered by the 90°E Ridge in the east, the Mid-Indian Ridge in the west and the Southwestern Ridge in the south. The basin has a variety of geological sub-environments maintaining a realm of five different sediment types [21, 23]. In this paper, we report the variation in response of bacterial community to contrasting physiography, geochemistry, and sedimentology. The two cores chosen for this study, namely, TVBC-26 and TVBC-08R were located at 10°S, 75.5°E and 16°S, 75.7°E respectively. The core TVBC-26 lies in the terrigenous sediments from the northernmost part of the Basin with illite, kaolinite and chlorite constituting ~90% of its clay minerals [23]. Pelagic red clays from the southernmost part of the Basin dominate the location of core TVBC-08R.

## Materials and Methodology

The samples for the present study were collected and processed on-board C.V. *Akademic Boris Petrov* Cruise IV (March-May 2005), under the DOD (Department of Ocean Development) sponsored PMN-EIA-EVDC (Polymetallic Nodules-Environmental Impact Assessment-Environmental Variability Data Collection) project, from the Central Indian Ocean Basin.

The samples were collected with the help of USNEL –type box core of 50cmx50cmx50cm dimension. Sub-cores were collected using acrylic cores of 6.3cm diameter. The sediment cores were sectioned at 2cm intervals up to 10cm and at 5cm interval thereafter and collected in sterile plastic bags for further processing.

- *Total Counts of bacteria* were determined by acridine orange direct count [11] using Nikon 80i epifluorescence microscope.
- *Direct Viable Counts* for bacteria were determined according to Kogure et al [14]. For determining anaerobic viability a reductant like Na<sub>2</sub>S.9H<sub>2</sub>O at final concentration of 0.125% was added before incubation [30].
- *Heterotrophic retrievable counts on varying concentrations ZoBell Marine Agar* were assessed using three different concentrations of ZoBell Marine Agar namely, 100%, 50%, and 12.5%. The plates were incubated at 2°-5°C.
- *Autotrophic retrievable counts*: Nitrifying bacteria were enumerated on modified Winogradsky's media for ammonia oxidizers (NI) and nitrite

oxidizers (NII). Denitrifiers were enumerated on modified Leiske's medium. [3].

- *Autotrophic uptake of  $\text{NaH}^{14}\text{CO}_3$  by sediments*: Uptake of  $\text{NaH}^{14}\text{CO}_3$  to determine the autotrophic potential of the sediments was studied as per method modified from Tuttle and Jannasch [27] using  $\text{NaH}^{14}\text{CO}_3$  with  $5\mu\text{Ci/ml}$  activity (BRITS, Mumbai) at  $160\mu\text{l}/10\text{ml}$  of slurry. Suitable controls for sediment and labelled carbon were included.

## Results

The salient physiographic, sedimentological and geochemical characteristics of the cores TVBC-26 and TVBC-08R described in the previous research is summarized in tables 13. The nodule coverage, quality of the nodule, concentration of manganese, illite, kaolinite and chlorite, continental flux, and TOC are decidedly higher at TVBC-26. Though both the regions are

**Table1. Comparison between the physiographic characters of TVBC-26 and TVBC-08R**

<i>Characteristic</i>	<i>TVBC-26</i>	<i>TVBC-08R</i>	<i>Reference</i>
Location	10°S, 75°30'E	16°S, 75°30'E	ABP-IV cruise
Touch position	9°59'S, 75°30'E	16°01'S, 75°28'E	AAS-6 & ABP-4 cruise
Depth	5325m	5201m	
Abundant Nodule Type	Type A	Type B	[13]
Nodule grade	High, >4cm size, Mn/Fe ratio>5.0	Low, <4cm size, lower Mn/Fe	[7,19,20]
Sea-floor spreading rate	90mm/y	26mm/y	[17]
Stress due to tectonic activity	Tensional	Compressional	[17]
Temperature of bottom water	0.9-1.03°C	>1.03°C	[21]
Continental flux	More	Less	[20, 23]
Major influencing current	Antarctic Bottom Water	North Atlantic Deep-Water	[9, 28]

under the influence of Antarctic Bottom Water Current and the North Atlantic Deep-Water Current, TVBC-26 is influenced more from the former and TVBC-08R from the later [9, 28].

Consequently, the bacterial parameters show marked difference between the two regions and (Tables 47). In core 26 average (n=8) anaerobic viable count was one order higher, with natural viable cells (NVC) i.e., frequency of dividing cells occurring naturally, showing only a marginal increase (Table 4).

**Table 2. Comparison between some sedimentological characters of TVBC-26 and TVBC-08R**

<i>Characteristic</i>	<i>TVBC-26</i>	<i>TVBC-08R</i>	<i>Reference</i>
Sediment type	Siliceous ooze	Pelagic Red Clay	[20, 26]
Most abundant clay type	Illite	Montmorillonite	[23]
Al-montmorillonite (%)	28-32	33-38	[23]
Fe-montmorillonite	Present in 1 $\mu$ m and 1-2 $\mu$ m size fraction	Present in <1 $\mu$ m size fraction	[23]
Illite (%)	35-40	33-36	[23]

The cell-size was generally  $\leq 1\mu$ m in core TVBC-08R and ranged from 1.5-1.8 $\mu$ m in core TVBC-26. On an average retrievability of bacteria was in the same order ( $10^4$ .g<sup>-1</sup>) on different strengths of ZMA for TVBC-08R. Nitrifiers were an order higher in TVBC-08R while denitrifiers were marginally higher in TVBC-26. <sup>14</sup>C uptake at 5°C for 30hrs expressed as DPM values, were significantly higher for core TVBC-08R (Table 7).

**Table 3. Comparison between some geochemical characters of TVBC-26 and TVBC-08R**

<i>Characteristic</i>	<i>TVBC-26</i>	<i>TVBC-08R</i>	<i>Reference</i>
SiO <sub>2</sub> /Al <sub>2</sub> O <sub>3</sub>	6.7	4.5	[20]
Excess silica (%)	27.97	12.27	[20]
Biogenic silica (%)	10-35	5-10	[9]
Mn/Fe	Mn>Fe	Mn<Fe	[20]
TiO/Al <sub>2</sub> O <sub>3</sub>	0.033	0.034	[20]
Dissolved oxygen (ml/l)	4.2-4.3	4.1-4.2	[21]
C/N ratio	3-6	3-6	[9]
CaCO <sub>3</sub> (%)	0-0.5	0.5-1.5	[9]
Organic carbon (%)	0.25-0.35	0.15-0.20	[9]

**Table 4. Direct bacterial counts using epifluorescence microscopy**

Direct bacterial counts	Average bacterial counts $g^{-1}$ dry sediment (n=8)	
	TVBC-26	TVBC-08R
Total counts	$7.2 \times 10^8 \pm 4.3 \times 10^8$	$1.4 \times 10^9 \pm 4.4 \times 10^8$
Naturally viable counts	$9.1 \times 10^7 \pm 3.6 \times 10^7$	$7.2 \times 10^7 \pm 4.3 \times 10^7$
Aerobic viable counts	$2.3 \times 10^8 \pm 1.8 \times 10^8$	$2.6 \times 10^8 \pm 1.3 \times 10^8$
Anaerobic viable counts	$1.3 \times 10^8 \pm 7.9 \times 10^7$	$3.9 \times 10^7 \pm 1.3 \times 10^7$

**Table 5. Heterotrophic retrievability of TVBC-26 and TVBC-08R, on varying strengths of ZoBell Marine Agar.**

Concentration of ZoBell Marine Agar	Average bacterial counts $g^{-1}$ dry sediment (n=8)	
	TVBC-26	TVBC-08R
100%	$4.4 \times 10^3 \pm 1.2 \times 10^2$	$6.8 \times 10^4 \pm 5.7 \times 10^3$
50%	$4.7 \times 10^3 \pm 4.4 \times 10^3$	$3.3 \times 10^4 \pm 3.4 \times 10^3$
12.5%	$1.5 \times 10^4 \pm 2.3 \times 10^3$	$1.3 \times 10^4 \pm 2.3 \times 10^3$

**Table 6. Autotrophic retrievability**

Type of autotrophic bacteria	Average bacterial counts $g^{-1}$ dry sediment (n=8)	
	TVBC-26	TVBC-08R
Ammonia oxidizers (NI)	$1.2 \times 10^3 \pm 1.3 \times 10^2$	$3.0 \times 10^3 \pm 2.8 \times 10^4$
Nitrite oxidizers (NII)	$8.9 \times 10^3 \pm 6.6 \times 10^2$	$1.7 \times 10^4 \pm 2.3 \times 10^3$
Denitrifiers	$2.2 \times 10^4 \pm 2.2 \times 10^3$	$5.7 \times 10^3 \pm 3.8 \times 10^3$

**Table 7. Autotrophic uptake of  $NaH^{14}CO_3$  by sediments**

Core no	Average DPM value for $^{14}C$ uptake by sediments (n=8)
TVBC-26	$17 \pm 4$
TVBC-08R	$478 \pm 395$

## Discussion

Bacterial interrelations with nodule environments and their usefulness as indicators of environmental disturbance have been described earlier [4, 24]. The influence of geography, mineral composition, physicochemical parameters, and environmental disturbance on the dynamics of bacterial community are well established [12]. They also underpin most of the geochemical cycles that were previously thought to be driven by inorganic processes alone [10]. These bacteria may be viable and culturable, viable but non-culturable, non-viable and

non-culturable or even dead cells [8] and understanding some of these states would be useful in delineating their role as mediators of geochemical activities.

The metabolic activities of a microbial population in turn may be stimulated or inhibited by the composition and conditions of a substratum. Clay, for example, is one such important substratum, which influences microbial habitat physico-chemically [25]. While clays may enhance bacterial growth by acting as buffers, they might also hinder microbial growth and metabolism by adsorbing microbial nutrients and making them less available to the microbes [6]. Clay mineral distribution in the surficial sediments of the Indian Ocean indicates that the sediments are largely detrital in origin [15,16]. In general, illite, chlorite and kaolinite abundances decrease from north to south owing to continental flux [23]. Montmorillonite is highest in the pelagic clay suggesting derivation from the weathering of proximal ridge basalts [23].

Comparison of bacteriological, geochemical and sedimentological properties distinctly show that they could influence the bacterial abundance, distribution and physiology. Bacterial abundance in terms of total counts and retrievability encountered in the study are comparable to the previous reports [5, 18]. This work has examined the viability of bacteria for the first time in this region and surprisingly a significant fraction could be viable. Counts of FDC (frequency of dividing cells) showed that about 12% could be viable. Direct viable count estimates according to Kogure *et al* showed that as much as 32 % could be viable under aerobic condition in TVBC-26. Organic load in these sediments could be responsible for higher viability especially under anaerobic condition. The larger size of the bacteria could also be attributed to the higher load organic and terrestrial influence. Interestingly TVBC-08R has higher total counts probably due to lower grazing pressure from benthic animals. The average population of denitrifiers is about an order higher in the higher in TVBC-26, while that of nitrifiers is an order higher in the southern TVBC-08R. This is in accord with previous study where similar findings have been reported [31]. Reduced availability of utilizable carbon could perhaps trigger autotrophic potential in the microbial communities of TVBC-08R. Both <sup>14</sup>C uptake and relatively higher abundance of nitrifiers suggest that this could be possible [27]. In essence, TVBC-26 with a higher organic load is perhaps dominated by degradative/respiratory metabolism while TVBC-08R is relatively more autotrophic.

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