developed. A single keratodont measures 0.085 mm in length and 0.057 mm in breadth. Measurements of the tadpoles are presented in Table 2 and the structural details of the tadpoles in Figure 4.

Tadpoles are mainly surface feeders. Keratinized mouth parts help the tadpoles in utilizing the periphyton. Gut contents of tadpoles belonging to early stages were analysed to know the food items. Phytoplanktons belonging to the following genera were recorded from the gut contents. *Oscillatoria*, *Phormidium*, *Anabaena*, *Gloeotrichia* (Myxophyceae), *Achnanthes*, *Synendra*, *Tabellaria*, *Navicula*, *Melosira*, *Surirella*, *Nititzschia*, *Cymbella* (Bacillariophyceae), *Ulothrix*, *Chaetophora*, *Spirogyra*, *Zygnema*, *Desmidium*, *Volvox*, *Scenedesmus* (Chlorophyceae). Zooplanktons (*Philodina*, *Monostyla*, *Cyclops*) were encountered only in two cases. In one case a nematode was found. Occurrence of nematode in gut contents was earlier reported by Sahu[11] in the gut of *Rana alticola* tadpoles. Food items were identified with the help of Edmondson[12].

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A deep-sea bacterium with unique nitrifying property

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Sediment cores collected from the Central Indian Basin (10°1′–10°2′S, 75°59′–76°2′E) harboured a high population of nitrifying bacteria which ranged from 10^3 to 10^7 cfu per gram dry weight. This high density could contribute to the high nitrate concentration observed in these sediments. We report for the first time the unusual property of having both the phases of nitrification, i.e. $NH_4^+ \rightarrow NO_2^-$ (Phase I) and $NO_2^- \rightarrow NO_3^-$ (Phase II) under normal and high pressure conditions in an autotrophic nitrifying bacterium isolated from the deep sediments cores at 5000 m depth (15–20 cm below the sea floor).

THE Central Indian Basin (CIB) is characterized by unusually high concentration of nitrate in the pore water of sediments, which are highly siliceous. The nitrate concentration generally ranged from 31.25 to 50 µg at N l^{-1} and occasionally reached 76 µg at Nl^{-1}, unlike the Arabian Sea where it ranged from 4.99 to 35.82 µg at N l^{-1}. Moreover, nitrate levels in the CIB sediments showed no decline with increasing depth[1,2]. Nagendernath and Mudholkar[2] hypothesized that this high concentration and constant value of nitrate in the sediments may be due to the intense nitrification process. This process has long been known to be restricted to aerobic or micro-aerophilic zone of water[3] and shallow sediments[4] and not to deep-sea sediment cores. The nitrification process is often deduced from the chemical profiles in water or from activity measurements[5]. Though Carlucci and Strickland[6] reported more than three decades ago that nitrifiers are widely distributed in the marine environment, the number of nitrifiers mediating this process has rarely been determined. Nitrification is generally carried out by known nitrifiers which either oxidize ammonia to nitrite (Phase I) or nitrite to nitrate (Phase II)[7]. The present work reports an observation of a deep-sea nitrifying bacterium capable of performing both the phases of nitrification.

Serially diluted samples from the sediment cores in the CIB (10°1′–10°2′S, 75°59′–76°2′E) were plated onto a mineral medium which is essentially a modified Winogradsky medium[8] with pure agar (Himedia, Mumbai) as gelling agent. The medium was substituted with ammonium sulphate at 2 mM (final concentration) or sodium nitrite at 0.5 mM (final concentration) as energy source and incubated for a period of 45 days at < 10°C. The colonies were enumerated and expressed as colony forming units (cfu) per gram dry weight sediment. Representatives from morphologically different colonies were isolated and checked for the purity and nitrifying ability (both phases) in liquid Winogradsky's mineral medium. Among the 101 isolates one (CIB12) was found capable of performing both the phases of nitrification and henceforth selected for further studies.

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Whole cell fatty acid methyl ester (FAME) analysis was carried out for the isolate with a standard column of 25 m × 0.2 mm methyl phenyl silicone-fused silica, fitted to a gas chromatograph equipped with flame ionization detector and integrator (Hewlett Packard, USA), to estimate the concentration of unsaturated fatty acids of the bacterial cells. G + C content of the isolate was determined according to Marmur and Doty[9].

The ability of the isolate CIB12 to nitrify chemosynthetically under pressure was demonstrated by conducting experiments under 400 atm. at 5°C. Preliminary experiments showed that measurable activity was obtained in 5–7 days of incubation. Hence all experiments were carried out for the above period. For high-pressure experiments a hydraulic pressure chamber (Tsurumi Suiki and Co, Model 02970, Japan) was used. The bacterial inoculum used for the experiment always contained 1.18 × 10^{10} cells l^{-1}. Experiments were also conducted at 1 atm. at 28 ± 2°C for comparison. Nitrifying activity can be measured either by ^{15}N technique or colourimetric assay (chemical assay). Bianchi *et al.*[10] had shown that the activity determined by chemical assay was comparable to ^{15}N method. For the present study we used only the chemical assay. The activity in terms of ammonia and nitrite conversion was estimated as described by Parsons *et al.*[11] and the results are expressed in terms of µg at N l^{-1}. The autotrophic ability of the isolate was further determined by assessing its ability to fix $NaH^{14}CO_3$ (specific activity of 5 µCi/ml; BARC, Mumbai). For bacterial enumeration, sub-samples from the above experiments were collected and preserved in 4% (0.22 micron filtered) buffered formalin, stained with 0.01% aqueous solution of acridine orange (Himedia, Mumbai) and enumerated under epifluorescence microscope (Olympus BHF) using a blue filter[12]. Experiments were repeated thrice and similar trends were noticed.

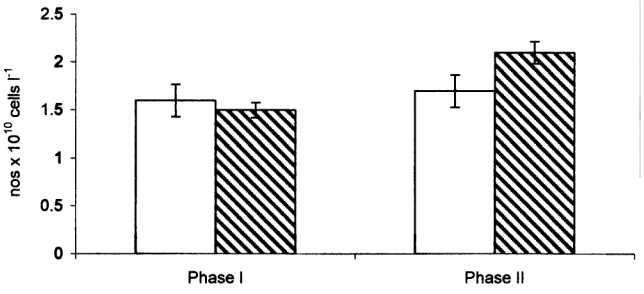

Figure 1. Bacterial counts of CIB12 after 7 days of incubation under normal (plain) and high (hatched) pressure. (Conditions as mentioned in Table 1.)

The nitrifying bacterial population generally ranged from 10^3 to 10^7 cfu per gram dry weight sediment. About 85% of 101 isolates retrieved from the plates oxidized only one phase, i.e. either ammonia to nitrite or nitrite to nitrate under autotrophic condition.

Isolate CIB12, which was found to carry out both the processes under strictly autotrophic conditions, had distinct colony morphology in being round, flat and very transparent, measuring 0.5 mm in diameter. Cells were gram-negative, non-motile rods measuring 0.9 µm × 0.45 µm in size. It was oxidase and catalase negative and utilized glucose oxidatively. High percentage of branched unsaturated fatty acids indicated that the isolate was baroduric in nature[13]. Based on the scheme of Oliver[14], it was found that the isolate could belong to the genus *Acinetobacter*. Reported G + C content for *Acinetobacter* ranges from 38 to 45 mol% (ref. 15). However, in this isolate the G + C content was only 26 mol% which is much below the reported values of 48 to 61 mol% for nitrifiers[16]. Experimental studies with the isolate showed that they could grow and nitrify at both 1 atm. and 400 atm.

There was an increase in the number of cells over the 7-day period (Figure 1), indicating normal growth. The increase in the number of cells under phase I was found to be 23% under pressure compared to 26% at normal pressure. In the case of phase II, the corresponding values were 78 and 45%. However, increase in the number of cells under normal and pressure conditions was not statistically significant.

Ammonia-oxidizing activity was about three times greater under 400 atm. at 5°C than at 1 atm. at 28 ± 2°C (Table 1). Nitrite-oxidizing activity was higher under both the experimental conditions. Nitrite-oxidizing activity was at least four times higher than ammonia oxidation, but the strain performed better at 1 atm. at 28 ± 2°C. The experiments demonstrated that the strain oxidized ammonia under simulated condition of high pressure and low temperature, indicating that it could be functional in the deep-sea sediments. In the deep sea, nitrification may not be restricted to the sediment–water interface[17] alone, but

Table 1. Nitrification and autotrophic activities of the isolate CIB12

Condition	Nitrification activity (one week) (µg at N l^{-1})	Nitrification activity (d^{-1}) (µg at N l^{-1})	Specific nitrification activity (µg at N l^{-1} $cell^{-1}$ d^{-1})	Specific activity, $^{14}CO_2$ fixation (µg at C l^{-1} $cell^{-1}$ d^{-1})
Phase I				
400 atm. at 5°C	8.09 (± 0.15)	1.16 (± 0.02)	0.77×10^{-10}	1.90×10^{-12}
1 atm. at 28 ± 2°C	2.81 (± 0.31)	0.40 (± 0.02)	0.25×10^{-10}	3.15×10^{-12}
Phase II				
400 atm. at 5°C	34.39 (± 1.82)	4.91 (± 0.35)	2.33×10^{-10}	11.99×10^{-12}
1 atm. at 28 ± 2°C	36.18 (± 0.07)	5.16 (± 0.01)	3.00×10^{-10}	15.92×10^{-12}

could percolate deep down where oxygen or any other electron acceptor is available. The pore water chemistry from this area showed consistently high Eh values (with more than + 300 mV)[1], indicating oxidizing conditions. This was mainly due to the influx of oxygenated (4.2 ml l^{-1}) Antarctic bottom water (AABW) above the sea floor[18]. The conditions were therefore conducive for the proliferation and activity of nitrifying bacteria. The cells were also able to fix $^{14}CO_2$ under both the experimental conditions showing chemolithotrophy (Table 1). From the above study it is evident that the strain not only oxidizes ammonia and nitrite under both experimental conditions, but also is able to carry out the phase I activity better under pressure. However, the specific activity was almost close to the reported values[19] for *Nitrosococcus oceanus* and *Nitrosomonas marina*, which ranged from 0.22 to 20×10^{-13} µg at N l^{-1} $cell^{-1}d^{-1}$.

Though Ward[17] had recently emphasized that there are no available reports on the ability to carry out both the phases of nitrification by a single organism, we found that in CIB region, especially at 15–20 cm below sea floor (bsf), the nitrifiers completely oxidized ammonia to nitrate. The phylogeny of nitrifiers shows them to have descended from a common photosynthetic ancestor. The ammonia oxidizers are found in the beta and gamma subdivision of the Proteobacteria, whereas the nitrite oxidizers are found in the alpha, delta and gamma subdivisions[20]. The photosynthetic bacteria are not only autotrophic, but also organotrophic. Besides they are able to oxidize reduced sulphur compounds to sulphur and then completely to sulphate. Hence it is speculated that this group of bacteria could be the missing nitrifers that have retained the ability to oxidize reduced nitrogen compounds completely to nitrate. These forms could be the ancestors from which either the ammonia or nitrate oxidizers have descended. As possessing both the systems for a chemolithotrophic mode of growth is too expensive for the cell, the descendents may have retained only one of these traits. Besides, nitrite oxidizers have been shown to augment chemolithotrophic lifestyle with heterotrophic metabolism of simple carbon substrate[17]. Retaining both the traits enables them to exploit unique niches several centimetres bsf, where carbon or energy sources could be much less than those at the surface or overlying waters. Studies on its molecular taxonomy would indicate its identity and lineage. Further, work on these lines is underway.

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