

Assemblage of benthic diatoms and culturable heterotrophs in shallow–water hydrothermal vent of the D. João de Castro Seamount, Azores in the Atlantic Ocean

C. Raghukumar^{1,*}, C. Mohandass¹, F. Cardigos², P. M. D’Costa¹, R. S. Santos² and A. Colaço²

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

²IMAR-Department of Oceanography and Fisheries, University of Açores, Cais de Sta Cruz, 9901-862, Horta, Portugal

The shallow-water hydrothermal vent of D. João de Castro Seamount in the North Atlantic, between the Azorean islands São Miguel and Terceira, is characterized by yellow and white zones which are distinct in physical and chemical characteristics. The white zone is characterized by high content of H₂S, CH₄, H₂, Pb and Co, and temperature of ~37°C. The yellow zone is characterized by high concentrations of Fe, Ba and Mn and temperature of ~60°C. In this recently described vent site, we have studied species diversity of benthic diatoms and heterotrophic microorganisms and their metal tolerance. Species diversity of benthic diatoms was marginally higher in white than the yellow zone. A higher number of heterotrophic organisms was isolated from the yellow zone than the white zone. Most of the culturable heterotrophic bacterial isolates showed better growth in the presence of Fe, Mn and Pb than in their absence. Here we report the presence of culturable filamentous fungi and thraustochytrid protists from the shallow-water hydrothermal vent. Growth and protease production in one of the thraustochytrid cultures, were not affected by the addition of Fe, Mn and Pb to the culture medium. The presence of such metal-resistant bacteria and thraustochytrids may suggest their adaptation to survive in metal-enriched waters of hydrothermal vents.

Keywords: Bacteria, fungi, hydrolytic enzymes, shallow-water hydrothermal vent, thraustochytrids.

THE D. João de Castro Seamount (DJCS) located in the North Atlantic, between the Azorean islands São Miguel and Terceira (38°13.3’N, 26°36.2’W), lies on the Terceira Rift (Figure 1). This rift is considered to be the world’s slowest spreading plate boundary¹. The area of the DJCS, shallower than 50 m is approximately 0.35 km², with 0.011 km² showing hydrothermal activity. The vent fluids have much higher trace metal and gas concentrations than

the ambient North Atlantic sea water². The shallow-water submarine hydrothermal vent is characterized by the presence of streams of gas bubbles (Figure 2 a). The DJCS lies close to other vent sites on the mid-Atlantic ridge with a diverse range of vent fauna, the closest being Menez Gwen³ at a distance of ~410 km and at 850 m depth.

At the DJCS, two distinct vent areas with different physical and chemical characteristics have been identified². The ‘white area’ has vent orifices with white bacterial mats, while the ‘yellow area’ has yellowish coloured vent openings (Figure 2 b). The temperature in the white area ranges from 26.5 to 43°C, and the fluids show higher concentrations of H₂S, CH₄, H₂, Pb and Co compared to fluids in the yellow area. On the other hand, fluids in the yellow area of the vent² show higher concentrations of Fe, Ba and Mn, and temperatures up to 63°C. The macrobiological communities of the DJCS in relation to vent-fluid chemistry and sediment composition have been described^{2,4}. No typical hydrothermal vent fauna were found at this site.

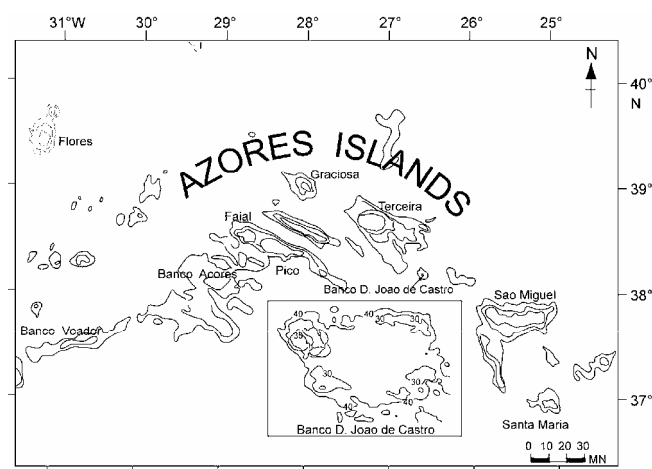


Figure 1. Sampling site of the shallow-water hydrothermal vent of the D. João de Castro seamount (DJCS) in Azores.

*For correspondence. (e-mail: lata@nio.org)

Microbial investigations on the hydrothermal vents mostly dealt with the selective isolation of chemosynthetic and thermophilic bacteria^{5,6}. Although the trophic role of heterotrophic bacteria in the vent ecosystem is not completely understood, a role in detoxification processes by these microorganisms associated with invertebrates has been postulated^{7,8}. Viable counts and reports of heterotrophic metal-oxidizing bacteria associated with polychaetes confirm the importance of these in the vent systems⁹. Microbial diversity of a few shallow-water hydrothermal vent ecosystems such as Matupi Harbour in Papua New Guinea¹⁰, Vulcano Island of Italy⁶ and Milos Island from Greece⁵ has been investigated in detail. The present work reports the diversity of benthic diatoms and culturable heterotrophic bacteria, mycelial fungi and thraustochytrid protists in the DJCS site. The effect of metal ions on the growth of heterotrophic bacteria was investigated to assess their susceptibility/resistance to these. The effect of metal ions on growth and protease production in one of the thraustochytrid isolates was examined to understand their effect on microbial processes in the hydrothermal vent ecosystem.

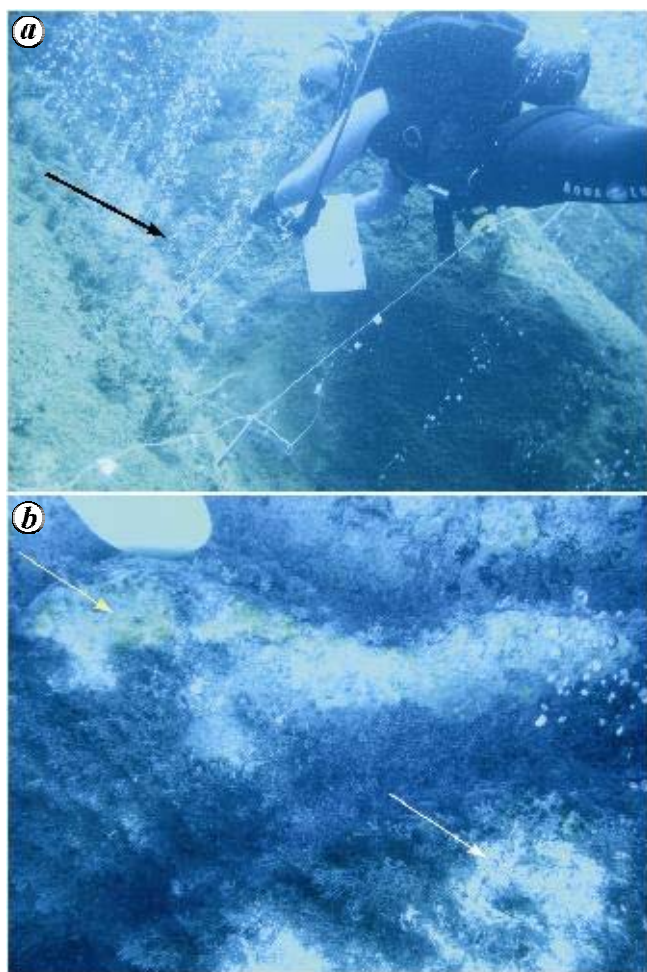


Figure 2. *a*, DJCS hydrothermal vent site. Arrow shows the presence of bubbling. *b*, Yellow (yellow arrow) and white (white arrow) zones at the sampling site.

Material and methods

Sampling

Sediment samples from the white and yellow areas (two samples each) of the vent site were collected from the DJCS hydrothermal vent during September, 2005 by SCUBA diving. The sediment samples were fixed in Lugol's solution¹¹ for enumeration of diatoms and in buffered formalin (5% V/V) for bacteria and stored in the dark at 4°C on-board the research vessel *Arquipélago*. The samples were transported in ice packs to Goa and all further analyses were carried out at the National Institute of Oceanography, Goa.

Total counts of bacteria

An aliquot of sediment samples (two samples each) from the yellow or white zones fixed in buffered formalin was appropriately diluted, sonicated in an ultrasonicator in order to free sediment-bound bacteria (3×10 s, 10 Hz; Sonic & Material Inc, CT, USA), filtered over black nucleopore filters of 0.22 μ m pore size (Millipore, USA) and stained with 0.01% aqueous solution of acridine orange for 5 min. Bacterial cells were counted using an epifluorescence microscope (Olympus BHF, Japan). Bacteria were counted from ~20 microscope fields. The final numbers were expressed as acridine orange direct counts (AODC) per ml sediment slurry¹².

Diversity of benthic diatom populations

Appropriately diluted sediment samples from the yellow and white zones (two samples each) were observed under an Olympus microscope for the presence of diatoms. The diatoms were identified using the standard morphological parameters¹³ and photographed.

Microchemical test for iron

For detecting the presence of iron in the sediments and around diatom cells, Prussian blue test was applied¹⁴ using aqueous potassium ferrocyanide in 1% HCl. Ferrocyanide produces a deep blue colour with ferric iron. Absolute care was taken to avoid using any iron-containing objects during this staining procedure.

Isolation of bacteria, thraustochytrid protists and fungi from sediments

Isolation of bacteria, fungi and thraustochytrids was carried out in Horta, Portugal and further work with these cultures was carried out at Goa. Viable aerobic heterotrophic bacteria from sediments (two samples each of yellow and

white zones respectively) were isolated on nutrient agar medium (Hi-Media, Mumbai, India) of 2.8% concentration prepared with sea water. The plates were incubated for 1 week at 37°C (the incubator adjusted to 60°C did not work in the shore laboratory at Horta, Portugal), and the results were expressed as colony forming units (CFUs) per ml sediment slurry. After isolation and purification of individual bacterial cultures, they were tentatively identified by biochemical tests performed in duplicate using 1-day-old cultures¹⁵. Each strain was examined for 30 separate characteristics. Utilization of various carbon sources (12 sugars) by the purified cultures was tested using the Hi-Media kit (Mumbai, catalogue No. KB 009).

Thraustochytrid protists were isolated by the baiting technique, wherein sediment (~0.1 g from two samples each of the yellow and white zones respectively) was added to sterile sea water with pine pollen¹⁶. Purified cultures of thraustochytrids were maintained in modified Vishniac agar medium containing 0.01% glucose, 0.01% peptone, 0.05% yeast extract, vitamin mix and 1.5% agar in sea water, according to Vishniac¹⁷. Fungal cultures were maintained on malt extract agar medium (Hi-Media, Mumbai) prepared in sea water.

Hydrolytic enzymes of fungi, thraustochytrids and bacteria

Fungi and bacteria for specific enzymes were isolated on malt extract agar and nutrient agar media respectively, supplemented with starch, carboxymethyl cellulose, xylan, Tween 80 (polysorbate 80 polyoxyethylene) and skimmed milk powder at 1% concentration (W/V). For detection of amylase, cellulase, xylanase, lipase and protease, the fungal, bacterial and thraustochytrid cultures were grown in appropriate agar media containing the above carbon sources. Amylase was detected by the presence of clear zone around the colonies after flooding the culture plates with Lugol's iodine solution. Lipase activity was detected by the appearance of white precipitate around the colonies. Clear zone in xylan, cellulose and skimmed milk powder-supplemented culture plates confirmed the presence of xylanase, cellulase and protease activity respectively¹⁸.

Effect of metals on bacteria and thraustochytrids

Effect of metal ions, iron and manganese at a final concentration of 1 µM, and lead at 1 nM (concentration of Pb above this was found to be inhibitory) final concentration was tested on the growth of bacterial cultures isolated from DJCS vent site. Zobell's marine broth supplemented with various concentrations of filter-sterilized solutions of FeCl₃, MnO₂ and Pb(CH₃COO)₂·3H₂O was inoculated with bacterial cultures having initial OD of 0.01–0.02 at 600 nm. Medium without metal supplement was used as

the control. Absorbance of the cultures was recorded at 600 nm every 24 h interval till the 8th day to assess the growth of each bacterial isolate in the presence and absence of metal ions. From the growth curve of each isolate, in each medium the following parameters were determined: the highest OD (OD_{max}) obtained, the day on which OD_{max} was attained, the time of first detectable OD change, the day when 90% of OD_{max} was reached (OD₉₀), and the day when maximum slope or growth rate was obtained¹⁹. The data represent mean of three replicates.

The effect of these three metal ions on growth and protease enzyme production on thraustochytrid isolate (2a, *Ulkenia* sp.) was tested at two different concentrations. The biomass of thraustochytrid was estimated on day-6 and expressed as mg dry weight per ml. Protease enzyme was estimated in the culture supernatants on day-6. In another set of experiments, the effect of these metals on protease activity per se was estimated by adding different concentrations of various metals to the culture supernatant during enzyme assay. The culture supernatant obtained from cultures grown without the addition of any metal ion was used for this purpose. In the control treatment, metal ions were not added during the reaction. The data represent mean values from three replicates.

Characterization of protease from the thraustochytrids culture 2a

The optimum pH, temperature and stability of partially purified protease from culture 2a were determined. For partial purification of the enzyme, about 50 ml of the culture filtrate from 6-day-old culture was concentrated to 5 ml by ultrafiltration through Amicon YM10 membrane (Millipore, USA) at 4°C. The effect of various concentrations of NaCl on the activity of partially purified enzyme was also determined. Protease was assayed according to Damare *et al.*²⁰ using azocasein (Sigma Chemicals, USA) as the substrate. Three replicates were maintained for all the assays.

Results

Diversity of benthic diatoms at the vent site

The seamount area was rich in macroalgae. Sediments around the vent at the DJCs were basically of two distinct types corresponding to white and yellow zones, based on the bottom colour around the vents and their physical and chemical parameters (Table 1). The yellow zone contained amorphous material harbouring several species of diatoms (Table 2), all of them covered with golden-brownish granular material (Figure 3a). The diatom cells and sediments from the yellow zone stained blue with Prussian blue stain indicating the presence of iron (Figure 3b and c). Microscopic observation of the unfixed sample

showed the presence of several gliding diatoms in a mucilaginous matrix, which stained positive for iron. Diatom cells were present in the white zone also (Table 2), but were not covered with yellow granular material. Some of the diatom species in the white zone were larger in size than the normal size reported for these species (Table 2). Species diversity was marginally higher in the white than in the yellow zone.

Diversity of heterotrophs at the vent site

The white zone was covered with white fluffy mats having filamentous, coccoid and sulphur bacteria containing glistening granules of sulphur (Figure 4 a). Black filamentous structures were commonly observed in the yellow zone (Figure 4 b and c). Melanized filamentous fungi were detected directly in sediments from the yellow zone (Figure 4 d). Bacterial cells and melanized fungal filaments were embedded in the yellow granular matrix. The average bacterial size in the coastal sediments was 1.1 μm , whereas those from the white and yellow zones measured 3.2 μm ; thus vent bacteria were three times larger than the normal size reported for these species. Total counts (AODC) were higher in the yellow than the white zone, whereas (CFUs) of bacteria were higher in the white zone than yellow zone (Table 3). Among the total 23 heterotrophic culturable bacteria isolated from the two zones, *Pseudomonas* and *Alcaligenes* dominated. All the cultures were Gram-negative. All the 23 isolates utilized maltose, fructose, dextrose and trehalose. Lactose and raffinose were utilized by strain # 11 and #15, and only strain #1 utilized mellibiose.

The number of fungi and bacteria isolated in various enriched media was much higher in the yellow than in the white zone (Table 3). Almost equal number of thraustochytrids was isolated from white and yellow zones of the vent site using pine pollen baiting technique (Table 3).

Table 1. Characteristics of white and yellow zones at D. João de Castro Seamount (DJCS) vent site (adapted from Cardigos *et al.*²)

Parameter	White zone	Yellow zone
Depth (cm)	25.8	19.9
T ($^{\circ}\text{C}$)	35.8	61.3
pH	5.01	5.09
Sulphur (μM)	81.6	1.24
H ₂ (μM)	3183.5	84.4
CH ₄ (μM)	29.8	12.8
H ₂ S (μM)	22.5	29.0
Cu (μM)	0.04	0.03
Pb (nM)	3.6	1.9
Fe (μM)	8.8	89.2
Mn (μM)	0.5	6.8
Co (μM)	0.04	0.03
Cd (μM)	0.006	0.005

Hydrolytic enzymes of heterotrophs

The highest number of fungi was present in the plates with skimmed milk powder indicating the dominance of

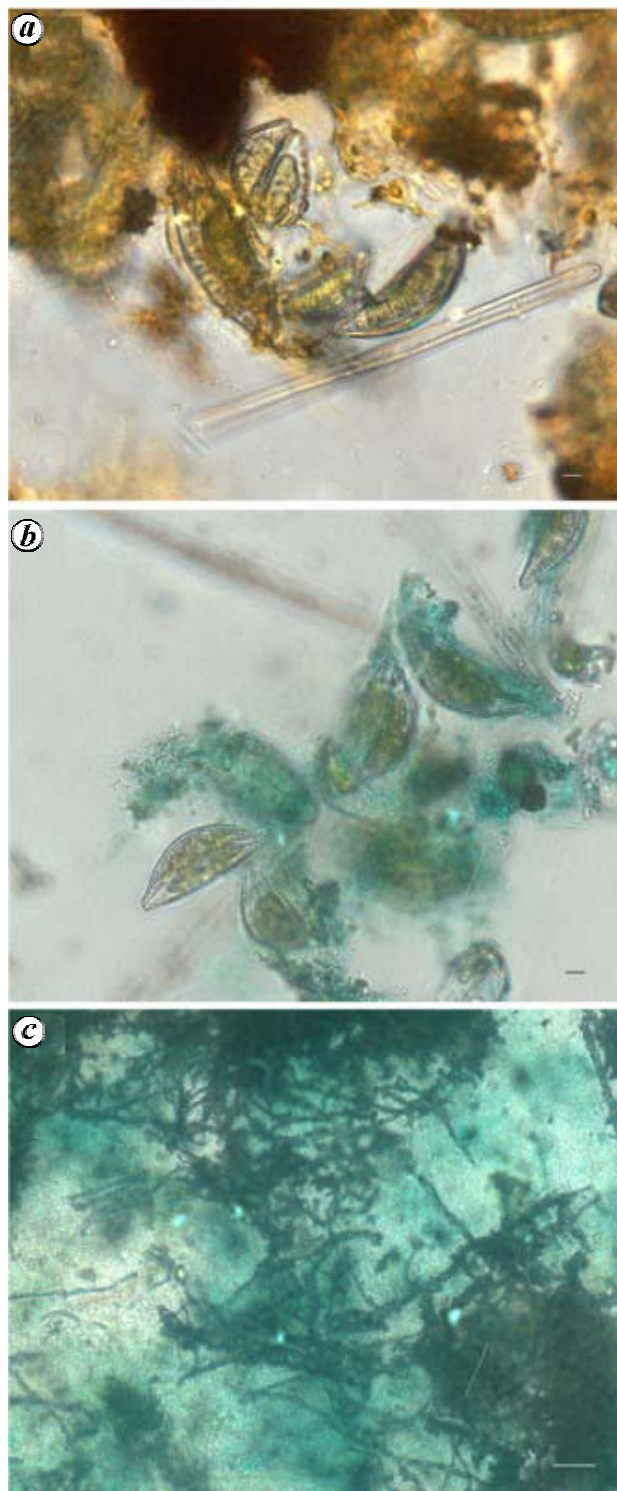


Figure 3. a, Yellow precipitate around diatom cells in the yellow zone; Bar = 10 μm . b, Yellow precipitate around diatoms stained positive for iron with Prussian blue stain; bar = 10 μm . c, Granular matrix around the filamentous bacteria (showing positive reaction for the presence of Fe with Prussian blue stain); bar = 5 μm .

Table 2. Assemblage of benthic diatoms at DJCS vent site

White zone	Yellow zone
<i>Rhabdonema adriaticum</i> (pennate diatom), 256.5 × 5.7 μm (normal size being 80 × 5 μm)	–
<i>Rhabdonema</i> sp. (pennate diatom)	<i>Rhabdonema</i> sp. (pennate diatom)
<i>Biddulphioid</i> sp. (centric diatom)	–
<i>Surirella</i> sp. (pennate diatom)	<i>Surirella</i> sp. (pennate diatom)
–	<i>Navicula</i> sp. (pennate diatom)
<i>Melosira</i> sp. (centric diatom)	<i>Melosira</i> sp. (centric diatom)
<i>Grammatophora marina</i> (pennate diatom)	<i>Grammatophora marina</i> (pennate diatom)
<i>Pleurosigma angulatum</i> (pennate diatom), 39.9 × 250.8 μm (normal size being 33.3 × 216.7 μm)	–
<i>Amphora</i> sp. (pennate diatom) 11.4 × 45.6 μm (normal size being 10 × 42 μm)	<i>Amphora</i> sp. (pennate diatom)
<i>Pleurosigma</i> sp. (pennate diatom)	–
–	<i>Licmophora</i> sp. (centric diatom)
<i>Pyrocystis</i> sp. (dinoflagellate) 250.8 × 39.9 μm (normal size being 131.8 × 36.4 μm)	<i>Pyrocystis</i> (dinoflagellate)
<i>Ceratium deflexum</i> (dinoflagellate)	–

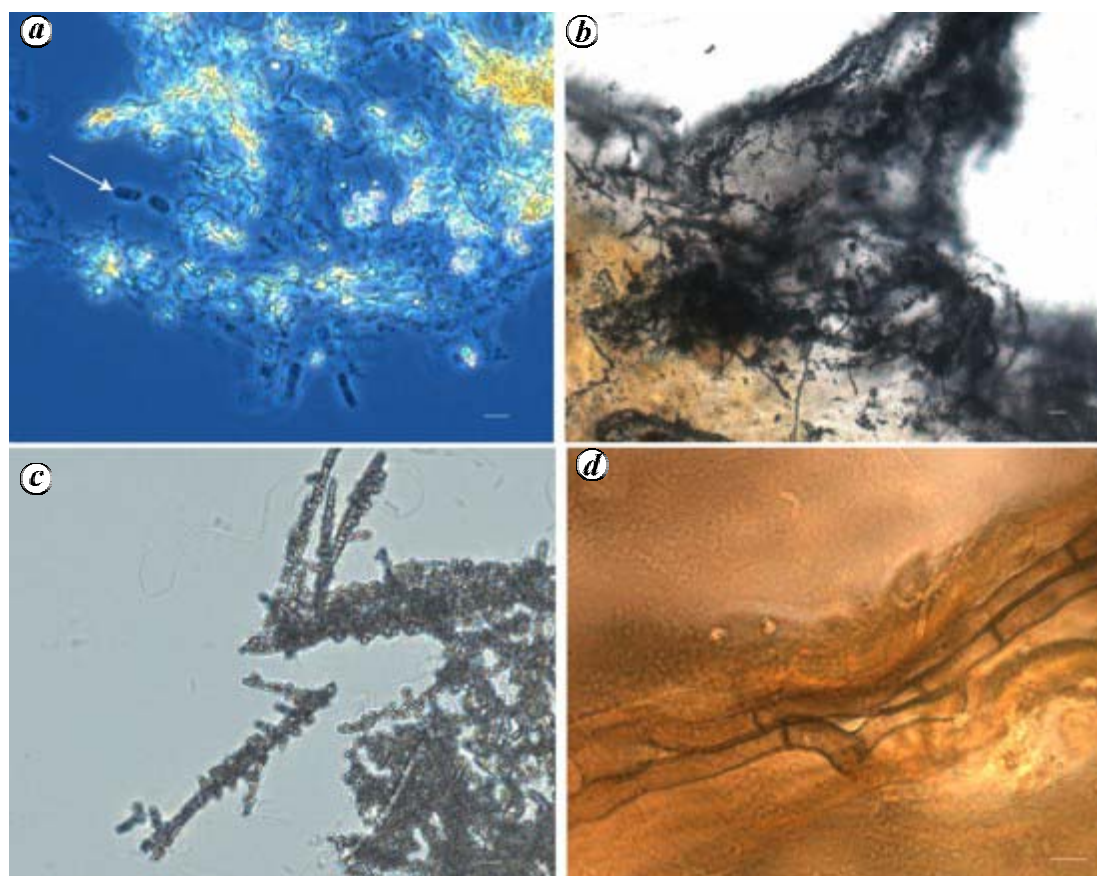


Figure 4. *a*, Filamentous bacteria and yeast-like cells (arrow) in the sediment mat in the white zone; bar = 5 μm. *b*, Filamentous structures within black precipitate from the yellow zone; bar = 5 μm. *c*, Rigid filamentous structures in the yellow zone; bar = 5 μm. *d*, Melanized fungal filaments with cross walls detected in the yellow zone; bar = 5 μm.

fungi with proteolytic activity, whereas a large number of bacteria (14 cultures out of total 23) showed lipolytic activity (Table 3). All of thraustochytrid cultures showed proteolytic activity (Table 3).

The partially purified protease enzyme from the thraustochytrid culture 2a (*Ulkenia* sp.) showed majority of activity between pH 9 and 10, and temperature 45°C and 50°C. The enzyme was most stable at pH 10.0, at 30°C. It was

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Table 3. Hydrolytic enzyme profiles of different microbial cultures grown on specific substrates. Numbers indicate positive cultures obtained in each carbon source

Media used ^a	White zone			Yellow zone		
	Fungi	Bacteria	Thraustochytrids	Fungi	Bacteria	Thraustochytrids
Cellulose	0	1	0	8	8	0
Xylan	0	0	0	4	6	0
Starch	1	2	0	3	8	0
Tween 80	0	3	0	3	11	0
Skimmed milk powder	0	0	5	16	6	6
Baiting ^b	0	0	5	0	0	6
Direct counts of bacteria (no. × 10 ⁸ ml ⁻¹) sediment slurry		155			221	
Bacterial CFUs/ml sediment slurry		4870			640	

^aCzapeck Dox Agar medium with different carbon sources as mentioned in the table was used. ^bSea water fortified with streptomycin–penicillin was baited with pine pollen for isolation of thraustochytrids.

Table 4. Effect of various metals on the growth of thraustochytrid culture 2a, protease production and its activity

Metal concentration	Growth (mg dry weight/ml)	Protease production (ACU/min/ml)	Protease activity ^b (ACU/min/ml)
Control ^a	1.0 (± 0.36)	338.2 (± 20.0)	551.5 (± 27.3)
Fe (15 µM)	4.86 (± 0.85)	400.0 (± 8.2)	553.7 (± 30.2)
Fe (150 µM)	1.40 (± 0.20)	299.5 (± 24.8)	527.5 (± 17.3)
Mn (7 µM)	3.06 (± 0.36)	346.6 (± 23.3)	586.6 (± 9.8)
Mn (70 µM)	1.56 (± 0.25)	355.5 (± 28.5)	548.0 (± 26.3)
Pb (4 nM)	2.80 (± 0.61)	349.7 (± 28.8)	580.0 (± 35.0)
Pb (40 nM)	2.54 (± 0.55)	316.0 (± 9.1)	554.2 (± 43.1)

^aWithout addition of any metal ions, ^bProtease activity was measured using azocasein substrate and expressed as (azocasein units) ACU/min/ml. The values are the mean of three replicates. One-way analysis of variance (ANOVA) revealed that all the parameters showed statistical significance between control and in the presence of different metal ions. *F*-value was 3.1, and *F*-critical value was 2.9 and *P* = 0.04.

100% active in the presence of NaCl in the concentration range 90–900 mM, equivalent to 0.5–5.0% salinity.

Effect of metals on thraustochytrids and bacteria

Growth of the thraustochytrid isolate 2a and activity of the enzyme protease were not affected in the presence of various metal ions tested at two different concentrations (Table 4). On the other hand, protease production decreased in the presence of higher concentrations of Fe. The effect of metal ions on growth and protease production as well its activity were statistically significant, as observed by One-way analysis of variance (Table 4).

Several of the bacterial isolates attained higher biomass in a shorter time period in the presence of Fe and Mn than in the nutrient broth without any metal enrichment. Only five of the isolates (isolate #3, 7, 13, 14 and 15) were not inhibited in the presence of Pb, and two of these (isolate #13 and 15) attained maximum growth earlier than the control cultures. A total of nine isolates (isolate #3, 4, 5, 8, 9, 13, 15, 18 and 22) showed increased growth in a

shorter duration of time in the presence of Fe than in its absence. Only six isolates (isolate #1, 5, 7, 15, 22 and 23) showed increased growth in the presence of Mn, whereas the rest of the isolates showed reduction in growth in its presence. A larger number of isolates showed OD_{max} within 3–5 days in the presence of metals, and maximum growth rate was achieved in the first one or two days (Table 5). Majority of the isolates in the presence of metals showed detectable OD change by the first day itself, and 90% of the maximum OD was reached within 3–5 days. The effect of metal ions on growth was statistically significant, as observed by analysis of variance (Table 5).

Discussion and conclusion

Benthic diatoms

The chemical composition and macroalgal diversity of sediments from the white and yellow zones of the DJCS shallow-water hydrothermal vent from the hyperslow-spreading Terceira Rift have been described in detail². The

Table 5. Analysis of growth kinetics of 23 heterotrophic bacterial cultures in the presence and absence of metal ions. A total of 23 bacterial cultures were tested for this experiment. From the growth curve of each isolate, in the absence or presence of various metal ions. The following parameters were determined – the day (1, 2 or 3–5 or 6–8) on which highest OD (OD_{max}) was attained, the maximum growth rate (1, 2 or 3–5 or 6–8 days) and the day (1, 2 or 3–5 or 6–8) when 90% of the OD_{max} (OD_{90}) was reached

Days	OD_{max}			Maximum growth rate ^a			First detectable OD change			OD_{90}		
	1–2	3–5	6–8	1–2	3–5	6–8	1	2	>3	1–2	3–5	6–8
Control	0 ^b	4	19	11	12	0	18	4	1	0	6	17
Pb	1	7	15	6	12	5	10	10	3	0	9	14
Fe	7	15	1	15	8	0	13	7	3	4	16	3
Mn	2	10	11	23	0	0	23	0	0	5	11	7
<i>F</i> -value		2.8			5.2*				12.4*		4.1	
<i>F</i> -critical		4.3			4.3				4.3		4.3	
<i>P</i> -value		0.11			0.03				0.002		0.06	
<i>df</i>		2, 11			2, 11				2, 11		2, 11	

^aMaximum growth rate indicated the day on which maximum OD difference between two consecutive days was observed. ^bThe figures represent number of isolates under each category. **F*-value greater than *F*-critical value obtained by ANOVA shows statistically significant difference in the number of isolates under each category. *df*, Degrees of freedom.

macroalgae *Sargassum* sp. and *Codium adherens* were found mostly near the yellow zone, whereas *Codium elisabethae* and *Padina* sp. were found growing near the white zone². We found only minor variations in the assemblage of diatom species in the two zones. However, we admit that these were one-time observations and need to be carried out seasonally for one or two years. A major distinction was the presence of iron precipitate around diatom cells in the yellow zone and its absence in the white zone. The metal-rich waters of the vent area support a rich diversity of diatom assemblage and some of them were larger in size than those in the coastal waters.

Heterotrophs

Filamentous structures that did not take up nuclear stain acridine orange were confined to the white zone alone. These structures appear to be similar to the filamentous sulphur formation reported in hydrothermal vents^{21,22}. Sulphur concentration in the white zone was several fold higher than in the yellow zone (Table 1). We do not know much about the rigid filamentous structures, heavily encrusted with brown to black precipitate confined to the yellow zone. These may possibly be associated with dissimilatory iron-reducing bacteria, which have been reported in a similar shallow vent site off Greece⁵. The lower number of CFUs from the yellow zone might be due to higher temperature in the yellow zone (~63°C). A similar distinct gradient in the bacterial population was noticed at a shallow hydrothermal vent near Milos Island, Greece⁵. The vent site at Milos has distinct concentric zonation of coloured surface deposits surrounding the gas outlet.

In spite of high AODC counts of bacteria, a much lower fraction of CFUs were obtained. The total counts do not differentiate between live and dead bacterial cells. There-

fore, a substantial fraction of total counts might consist of dead cells. Although it is well known that only a fraction of total counts is culturable²³, a large number of them requires special media²⁴, which were not provided in this study. The culture plates however were incubated at 37°C, the temperature prevalent in the white zone (the incubator with higher temperature stopped working in the shore laboratory during our sampling period).

Heterotrophic bacteria are generally ignored in the vent area and only the specialists are regarded to play a major role. Jeanthon and Prieur⁸ explored the role of heterotrophic bacteria associated with a deep-sea hydrothermal vent polychaete in the detoxification mechanism. We observed several isolates showing resistance to metals, and this might be an adaptive mechanism to survive in metal-rich waters. Microorganisms are known to employ a variety of mechanisms to adapt to the presence of heavy metal ions. One of these is the production of hydrogen sulphide, which may act as a detoxification process through precipitation of metal sulphides⁷. The yellow and white zones contained a reasonably higher concentration of H₂S, which might trigger precipitation of metal sulphides. The diversity of heterotrophic bacteria and their large size might indicate the positive effects of metal-rich waters of the vent site. Metal-tolerant bacteria²⁵ and flagellated protists²⁶ have been reported from deep-sea hydrothermal vents. Further examination of microbe–metal interactions in shallow-water hydrothermal vents is needed to understand the evolution of metal resistance in the natural microbial population.

Fungal filaments were detected only in the yellow zone. Culturable fungal colonies were also obtained mostly from the yellow zone. Here we report about culturable fungi and thraustochytrid protists from a hydrothermal vent site. Edgcomb *et al.*²⁷ have reported the presence of stramenopiles (thraustochytrids) and filamentous fungi by

SSU rRNA in sediments of a hydrothermal vent site in Guaymas Basin, Gulf of California. Tissue deterioration of a deep-sea mussel, *Bathymodiolus brevior* at a hydrothermal vent in Fiji Basin by a black yeast, *Capronia* has been reported recently²⁸. However, this was not cultured, but identified by the SSU rDNA sequence and detected in mussel tissue by fluorescent *in situ* hybridization²⁸. An ascomycete and unidentified fungal bodies associated with gills of an endemic gastropod found in hydrothermal-vent communities along the upper continental slope of the Gulf of Mexico have been reported²⁹. These reports indicate increasing awareness of fungal communities and their role in vent ecosystems.

Most of the fungi isolated from DJCS were melanized forms like *Aspergillus* and *Cladosporium* spp and dark brown to dark green, non-sporulating forms. Melanized fungal filaments were also directly detected in the sediments from the yellow zone at the DJCS site. Melanin is involved in absorbing free radicals and reactive oxygen species to protect the organisms from damage under extreme environmental conditions³⁰. Melanin is highly stable and does not degrade easily under harsh conditions. Therefore, presence of heavily melanized fungi in the yellow zone with temperature around 63°C, is interesting.

The growth of thraustochytrids in the presence of high concentration of metals might suggest their adaptations to vent conditions. Tolerance to high NaCl concentration and optimum activity of the protease in one of the thraustochytrid isolates at 45°C further indicate its strategy to participate in the degradation of detrital material around the hydrothermal vent site. As the vent site is dominated by macroalgae, sediments from yellow and white zones also contain a large amount of phytodetritus, which is a good source for these fungi and thraustochytrids to grow. High fungal biomass associated with phytodetritus of algae³¹ and seagrass³² is well documented. The role of filamentous fungi in degradation of the phytodetritus is further indicated by the presence of xylan, cellulose and starch-degrading activities of the isolated fungi. As the upper limit of temperature for thermophilic fungi is reported³³ to be 62°C, the presence of filamentous fungi in deep-sea hydrothermal vents is possible, and their role and biodiversity should also be considered in future studies on vent biology. Further, the presence of such microorganisms with proteolytic, lipolytic and other hydrolytic activities is known to be ecologically significant in areas of high nutrient concentrations⁷.

In conclusion, the DJCS vent site is characterized by a rich diversity of benthic diatoms, some of which show better growth and larger size in the presence of metal-rich waters. Assemblages of heterotrophic bacteria, a majority of them showing degradation of Tween 80, fungi and thraustochytrids with their proteolytic enzymes may play a vital role in the mineralization of organic materials in this nutrient-rich environment. The alkalophilic, halophilic and metal-tolerant protease enzyme from the thraustochy-

trid *Ulkenia* sp. may hold promise for biotechnological applications.

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Erratum

Natural parasitization and biological control: case of the coconut caterpillar

K. Muralimohan, Ramkumar and Y. B. Srinivasa
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On page 1481, Figure 3 was not properly printed. We reproduce below the figure with the caption.

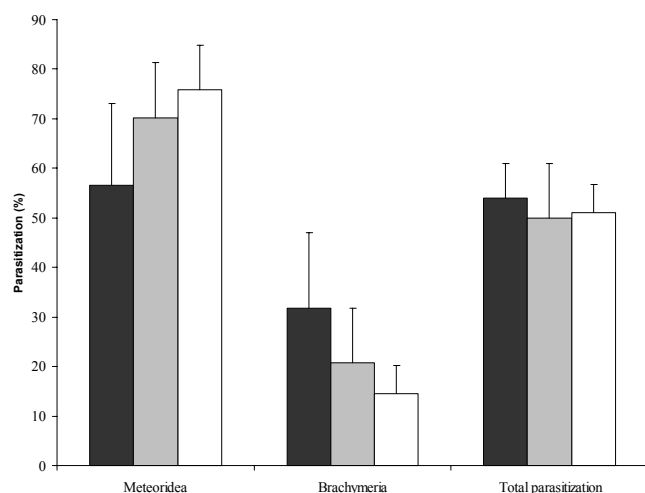


Figure 3. Pupal parasitization of *Opisina* by different parasitoids during summer (black bar), rainy season (grey bar) and winter (white bar) months of the year. Whiskers indicate one standard deviation.