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Microcosom investigation of Mn mobilization in basalt rock by potential bacteria R6 from Carlsberg ridge ecosystem

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ABSTRACT

The biomining can happen in basalt rocks from ridges which contains around 25% Mn in the form of different minerals and oxides due to deep sea hydrothermal activity. The prime goal of this experiment was to demonstrate Mn mobilization from natural Mn minerals and oxides using striping voltammetry from basalt near deep-sea hydrothermal vents (DSHVs) by a potential bacterial isolate R6 which was isolated from this environment. Natural basalt sample was collected from the carls berg ridge during ABP-36 cruise and was characterized by scanning electron microscopy (SEM) and X-ray diffractometry. Bacterial isolation was done in laboratory by spread plate method using 100uM Mn amended NA media plates. Isolated bacteria R6 (Accsession No. LK934696) and basalt sample were used in a laboratory batch experiment. The isolate R6 (identified as marine Bacteria *Imtechella halotolerans sp.*) and natural basalt rock were placed in 100% seawater in the presence and absence of an organic carbon supplement as 0.01% glucose (analogous abiotic and chemical controls systems were also included). This laboratory batch experiment was incubated in the dark at 28 ± 2 °C for 6 months and cell bio mass, pH, Eh and concentrations of mobilized Mn ions were measured over time. The presence of the bacteria induced the release of Mn from the basalt relative to the controls, especially with the addition of the organic carbon supplement. Bacteria was able to draw significant mobilization rate 27985.91 and 4797.37 µg g^{-1} d^{-1} with and without glucose added in biological experiment part when compared with abiotic and chemical controls. Bacterial colonies on the basalt fragments surfaces were examined by SEM which shows evidence of extrapolysaccherides secretion and mineral precipitation. The results of this study suggest that chemoorganotrophic bacteria are involved in the cycling of Mn mobilization in basalt near DSHVs.

Keywords: bacteria, basalt, mobilization, hydrothermal vent, marine, mineral

INTRODUCTION

Carlsberg Ridge (CR) in the Arabian Sea is a segment of the Mid-Indian Ridge near Rodrgius island which known as unique and extreme ecosystem due to low temperature, high pressure, very low organic matter and high availability of inorganic ions. All these environment characters leads to extreme kind of ecosystem and presence of microbes like bacteria those works as interlink between inorganic ions and fauna present in ridges near

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hydrothermal vent by chemosyenthetic activities where organic carbon fixing from inorganic ions (Das et al 2012). Hansell and Carlson, 1998 reported 42.8 µM DOC at water depths of 1000 to 4300 m which supports microbial oxidation as dominant process. But, DOC concentration varies from 70-80 µM during the northeast monsoon and 75-90 µM during the spring inter-monsoon period (Hansell and Peltzer, 1998) which may leads to microbial mobilization at same environment.

Ecologically and chemically Deep Sea Hydrothermal Vents (DSHVs) play an important role in several global chemical budgets by introducing an unique and important ecosystems (Seyfried and Mottl 1995; Van Dover 2000, and references therein). DSHVs formation is common phenomenon in ridges due to constructive margins of oceanic plates on the sea surfaces, where new oceanic lithosphere is continually generated due to rifting and are characterized by high heat flow and marked seismicity (Iyer et al. 2003). DSHVs are the source for hydrothermal solutions emerge from the seafloor. Direct Mixing of the hydrothermal floods with the surrounding seawater creates strong chemical and temperature gradients. Due to this chemical and temperature gradients, fluids become enriched in reduced metal ions which can be used by chemolithotrophic bacteria as a source of energy (Van Dover 2000). These chemolithotrophs works as base of the food chain in ridge ecosystem that also supports chemoorganotrophic microorgansms and diverse macrofauna. Bacteria play a potential role in the mobilization/weathering of minerals and oxides in many ways (Erlich 1996). Direct Mobilization of primary minerals can be induced by chemolithotrophic microbes and same time dissolution may happen from the precipitation of secondary minerals through enzyme-catalyzed oxidation or reduction (Lovley and Phillips 1988; Francis and Dodge 1990, 1991; Zachara et al. 1998). Producing ligands by chemolithotrophic or chemoorganotrophic microbes is indirect way to solubilize minerals (e.g., organic acids, metabolites, siderophores, polysaccharides) which form complexes with mineral-forming ions, which causes ligand-promoted mineral dissolution (Francis and Dodge 1990, 1991; Barker and Banfield 1996; Kalinowski et al. 2000; Liermann et al. 2000; Welch and Banfield 2002). Relatively few reports are available for Mn mobilization by bacteria in basalt particularly in marine environments or near DSHVs. Sujith et al 2014 have investigated Mn mobilization in ridge basalt rock by microbial community in the laboratory conditions (Sujith et al 2014) While Thorseth and colleagues were demonstrated microbial alteration of basaltic glass in the marine environment and in the laboratory. (Torseth et al. 1991, 1992, 1995a, 1995b; Fisk et al. 1998).

Hence, we hypothesize that along with certain environmental and chemical process for mineral deposition, immobilization by microorganisms associated with these basalts in ridges also have big contribution. Present study explains that bacteria are capable to mobilize Mn from basaltic minerals and oxides in their pure form of metal which strongly supports biomining in ridges. So, we used R6 an identitified culturable Mn(II)-oxidizing bacteria to check an interactive role in the mobilization of Mn in the ridge ecosystem. Same time Fortin et al. (1998) reported bacteria near DSHVs were coated with secondary Fe and Mn oxides and iron silicates, although it was not clear if the bacteria played a direct (enzymatic) or passive role in the formation of the precipitates (see also Juniper and Tebo 1995).

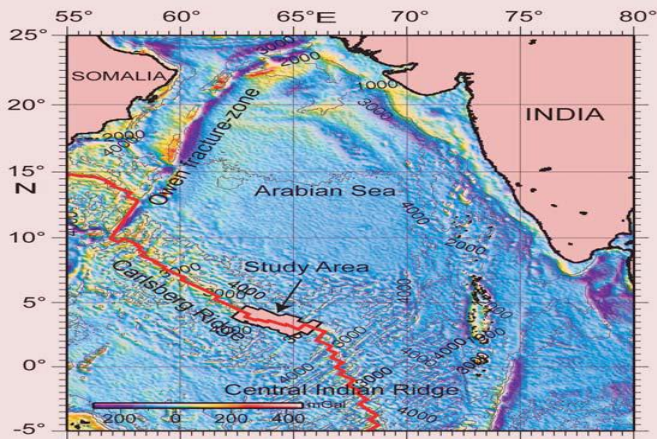
MATERIALS AND METHODS

Study area and sampling

The Carlsberg Ridge in the Arabian Sea is a segment of the Mid-Indian Ridge. It extends from near Rodrigues Island to the Gulf of Aden, trending Northwest to Southeast and separates the Arabian Sea in the Northeast from the Somali Basin in the Southwest (Fournier et al., 2008). The mean elevation of the Carlsberg Ridge crest is 2100 m and the water depth is 1800–3600 m (Mudholkar et al., 2000). A ridge rock sample bearing oxide coating was collected at 3°39.718N (lat) and 63°49.922E (long) (Fig. 1) during the Akademic Boris Petrov cruise 36

(2009), using a chain-bag dredge at a water depth of 3390 m.

Figure 1. Map of the study area (courtesy



Kamesh Raju et al. 2008 and Sujith et al 2014). The filled circle in green depicts the location of basalt sampling.

Characterization of the natural Mn oxides coating on basalt

Basalt fragments bearing oxide coatings of dimensions 0.52–3.83x0.66–3.71 mm (n = 10) were aseptically removed using a sterile scalpel and rinsed with phosphate buffered saline (300 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 1.7 mM NaH_2PO_4, pH 7.0) before microscopic analyses. They were air-dried, mounted on metal stubs using conductive adhesive tape and then sputter-coated with Au using a SPI-Module sputter coater. The edges were coated with conductive silver to eliminate any possible charging. Using a JEOL JSM-5800 scanning electron microscope (SEM) the surface texture and microbial load on basalt surfaces were observed at different magnifications.

Oxford INCA 5431 Energy Dispersive Spectrophotometer (EDS) in conjunction with SEM was done on randomly chosen points of the fragments at an accelerating voltage of 15 kV for determining the composition of the rock. The solid phase concentration of Cu was determined from 50 mg of powdered rock fragments by closed vessel digestion method (Roy et al., 2007). The determination of Mn was carried out by adsorptive stripping voltammetry in interface with 797 VA computrace (Metrohm, Switzerland) in a differential pulse mode according to Colombini and fuccoso (1983). The analysis was quantified with two standard additions of appropriately diluted 10 mgL^{-1} concentrations of Mn^{2+} standard ($MnCl_2 \times 4H_2O$). The concentration of Mn in solution was then normalized per gram dry weight of rock after correcting for the blank. The mineralogy of natural Ferromanganese oxide was characterized by X-ray diffraction analysis (XRD) as described by Villalobos et al. (2003). For XRD analysis, oxide coatings were removed from a single large rock fragment using scalpel and then finely powdered using an agate mortar and pestle. The XRD patterns of the ferromanganese oxides were recorded in a Rigaku X-ray powder diffractometer using a monochromatic Cu Ka1 radiation (operating at 40 kV and 20 mA) and a scintillation detector. All samples were run in scan mode over a 2h range of 0–80° with a scanning rate of 1.2° min^{-1}. The interpretation of the peaks was done according to Burns and Burns (1977).

Table 1. Experimental set up to measure rate of Mn mobilization by Bacterial potential isolate R6 on crust at 1atm and 28±1ºC.

	Blank				Heat Killed		Azide		Experiment	
	B1	B2	B3	B4	HK1	HK2	AZ1	AZ2	E1	E2
Glucose 10% (mL) (final 0.01%)	-	0.6	-	0.6	-	0.6	-	0.6	-	0.6
Azide 1 M (mL) (final 15 mM)	-	-	1	1	-	-	1	1	-	-
Crust (g)	-	-	-	-	1	1	1	1	1	1
Sterile seawater (mL)	600	599.4	600	598.4	600	599.4	599	598.4	600	599.4

Experiment on the basalt associated microbial community

Mobilisation of Mn:

Though reduction refers to dissolution by change in oxidation state, we have used the word mobilization to depict both reduction and dissolution. The experiment to demonstrate the mobilization of Mn from oxide coatings on basalt by bacteria resident on it was conducted in 1000 ml screw-capped glass flask containing rock fragments of dimensions 0.85-9.08 × 0.88-6.99 mm and 600 ml of liquid medium (G+ as 0.1% glucose while G- stands for without glucose) with as given in Table 1. The experiment was incubated in triplicate at 28±2 °C in the dark for 30, 60, 90, 120,150 and 180 d. After every 30 d of incubation, tubes from experiment and respective controls were sacrificed for the analyses. The dry weights of the rock fragments were determined after drying to constant weight at 105 °C.

Microbial growth upon incubation:

The cells were counted from 20 µl aliquots of the samples immediately using the Neubauer counting chamber or were preserved with 2% final concentration of buffered formalin prior to counting. For every sample, 50 small squares at 400X magnification were counted using a Nikon 50i bright field microscope (Tokyo, Japan) for determining the average number of cells.

pH and Eh:

The pH measurements were carried out using Thermo Orion Triode 3-in-1 pH electrode and Eh (mV) using Thermo Orion Epoxy body sure flow, combination redox/ORP electrode. The above electrodes were calibrated using reference solutions prior to measurements to ensure accurate readings. The calibration of the pH meter (Thermo Orion 3-star benchtop pH/mV/Temp. Meter) was as per the protocol given in the user's guide, Thermo Electron Corporation (2005). The calibration for pH was done using buffer solutions of known pH 4, 7, and 10. For Eh, the potential of the platinum (Pt) electrode versus the Ag/AgCl reference electrode with KCl electrolyte in ZoBell's solution was measured as a function of temperature. The Eh (mV) readings of the samples were taken for 60 s followed by pH, in triplicate. Eh values (mV) were calculated as described in standard methods APHA AWWA WEF (2005).

Determination of Manganese in solution:

The Mn in supernatant was analyzed after centrifugation of samples at 5000rpm for 10 min at 4°C Sigma 3-k). The procedure for Mn analysis was as described under section 2.2. However, the digestion in this case was under UV instead of acid. To deduce the change in metal concentration 1 ml supernatant from each tube was diluted with 9 ml of Milli-Q water (18.2 Ω resistance) and then acidified to pH < 2 with 30% Suprapur HCl (Merck). The acidified samples were UV digested using high-pressure mercury lamp (705 UV Digester, Metrohm) in quartz cuvettes at 90 °C for one hour. The UV irradiated samples were analysed immediately after cooling or were stored in the dark at 4°C until analysis. The concentration of Mn in solution was then normalized for the volume of media used and per gram dry weight of rock fragments after correcting for the respective blanks.

Scanning electron microscope and X-ray analysis:

The increase in bacterial numbers, cell morphology and details like the presence of exopolysaccharides (EPS) and cell aggregates were visualized under SEM at different magnifications both before and after150 days of incubation. EDS in conjunction with SEM was done to observe the changes in composition of the surfaces during incubation. The details of the procedures followed are described in section 2.2.

RESULTS

Microscopic and X-ray characterization:

Natural basalts mostly showed black coloured coatings with rough surface, numerous pits, fissures and fractures as physical characters. EDS spot analyses indicated heterogeneity in chemical composition with rough surfaces particularly high in Fe-Mn oxides (Fig 2 A and B).

Figure 2. Basalt rock sample A) initial day and B) end day of experiment where crystal formation happen due to bacterial activity.

The thickness of the coatings vary from >1-3.6 mm with greater thickness on irregular surfaces and vice versa on regular surfaces. The solid phase Mn concentration in the rock bearing Mn-oxide varied from 56.16 to 288.69 mg g-1.

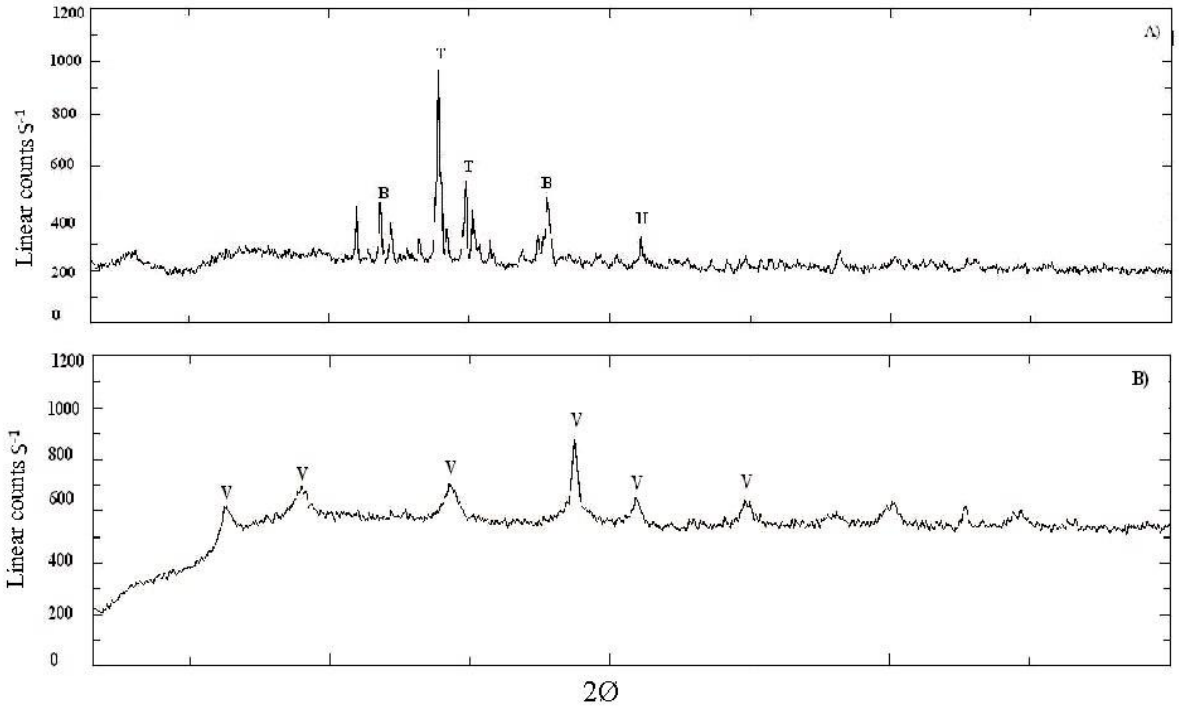
Mineralogical investigations showed todorokite [(Na, Ca, Mn)$_2$ Mn_5O_{12}. $3H_2O$] as the major and birnesite ($Na_4Mn14O_{27}.9H_2O$) as the minor Mn mineral. Halite (NaCl) was due to the background of seawater (Fig. 3 A and B).

Experiment on the basalt associated microbial community

Mobilization of Manganese:

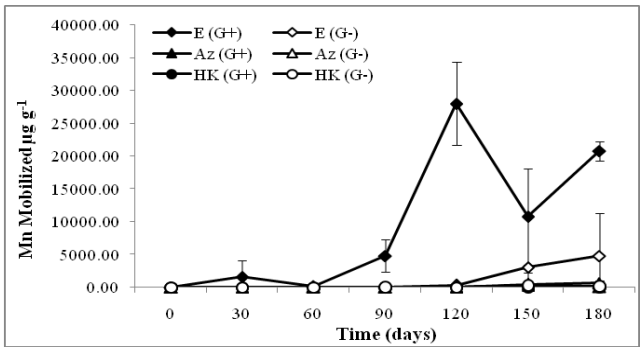
The mobilization rate of Mn was maximum at the end of 120 d incubation in 'G+' 27985.91 µg g^{-1} d^{-1} and 4797.37 µg g^{-1} d^{-1} in 'G-' media.

Figure 3. X-ray diffraction analysis results. A) Mn-oxide coatings removed from basalt surface and B) chemically synthesized Mn oxide (Peak labels B: birnessite, H: halite, T: todorokite and V: vernadite) (Sujith et al 2014).



(Fig.4). The experimental rates in 'G+' were 43 times and in 'G-', 22 times more than the respective azide poisoned controls. Compared to heat killed controls, the respective experimental rates in 'G+' were 478 times and in 'G-', 27 times more.

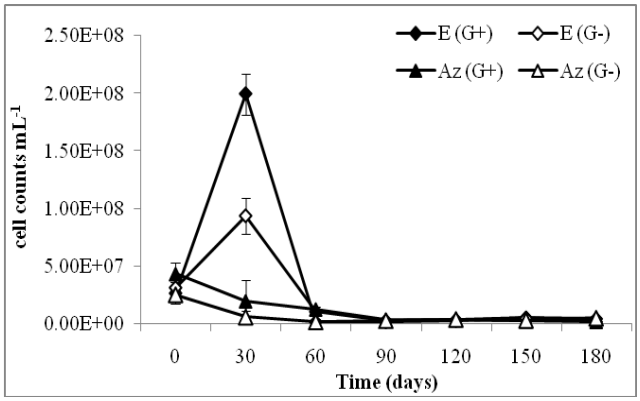
Figure 4. Mobilization rate of Mn from basalt by Bacterial potential isolate R6. The values in the figure are mean values ± SD, n = 3.



Bacterial growth:

TC increased by more than an order of magnitude in the presence of added glucose and less than an order of magnitude in the absence of added glucose (Fig. 5).

Figure 5. Variation in cell numbers in relation to the mobilization of Mn (E: experiment, Az: azide poisoned control, Hk: heat killed control, G+: with added glucose and G-: without added glucose).

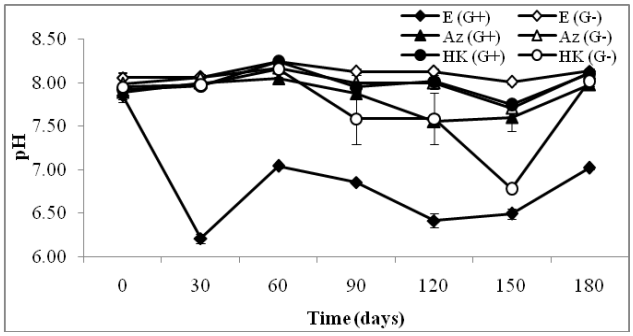


It ranged from 2.13 × 10^6 to1.99 × 10^8 cells g^{-1} in 'G+' and 2.27 × 10^6 cells g^{-1} to 9.37 × 10^7 cells g^{-1} in 'G-' incubations. The maximum cell counts in the presence of added glucose occurred on the 60 d of incubation (1.99 × 10^8 cells g-1) and in the absence of added glucose (9.37 × 10^7 cells g^{-1}) on the 60 d. In the azide poisoned control the counts ranged from 2.05 × 10^6 to 4.37 × 10^7 cells g^{-1} in the 'G+' and 2.92× 10^6 cells g^{-1} to 2.54 × 10^7 cells g^{-1} in the 'G-'.

Variation in pH and Eh:

Notable difference in pH and Eh was not perceptible between the experiments and Corresponding controls. The pH decreased from 7.85 to 6.21 in the 'G+' and from 8.24 to 8.01 in the 'G-' (Fig. 6).

Figure 6. Variation in pH in relation to the mobilization of Mn (E: experiment and Az: azide poisoned control, G+: with added glucose and G-: without added glucose).



The Eh shifted from negative to positive redox potentials at the end of incubation (60 d). It varied from -69.96to 126.2 mV in the 'G+' and -68.63 to 106.88 mV in the 'G-' incubations (Fig. 7).

Figure 7. Variation in Eh (mV) in relation to the mobilization of Mn (E: experiment and Az: azide poisoned control, G+: with added glucose and G-: without added glucose).

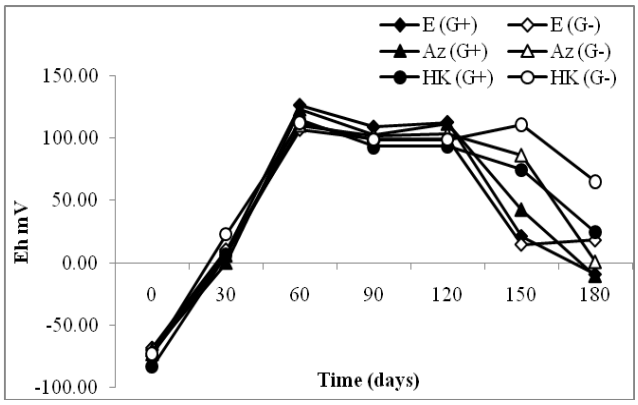


Figure 8. SEM images of R6 bacterial cells in glucose amended medium and associated with basalt. Bacterial cells Image initial day A) control and B) experiment. SEM Bacterial image associated with basalt final day C) control D) experiment.

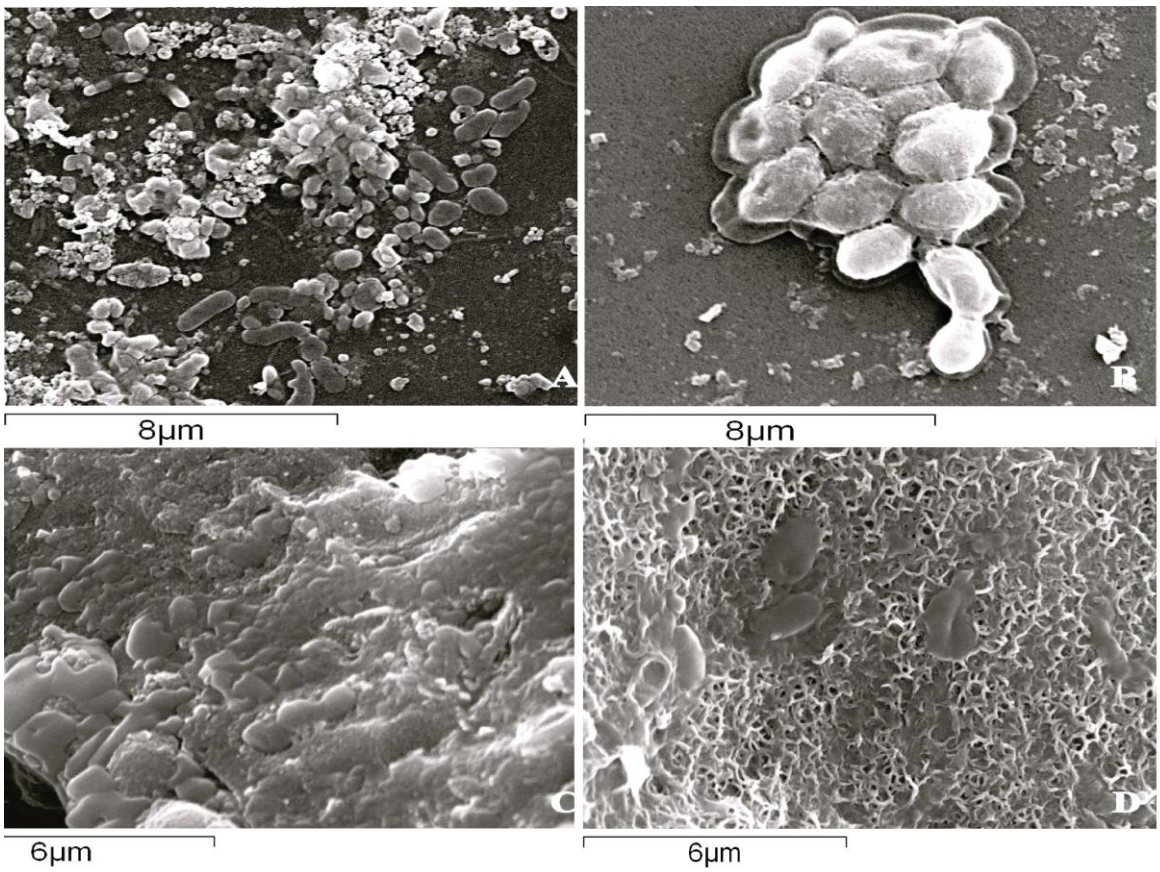
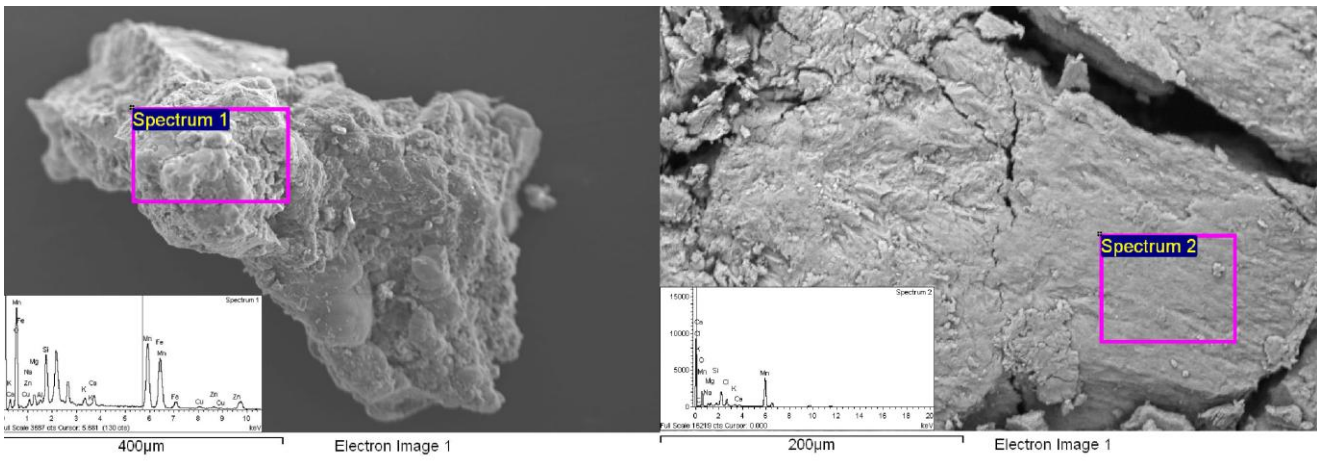


Figure 9. SEM images show the surface texture and EDS spectra. The chemical composition of basalt fragments before (A) and after incubation (B) for 150 d. The rough surface show higher concentration of Mn relative to smooth surface and tend to decrease with time on rough surface.



SEM and EDS analysis:

Compared bacteria R6 on 0 day and 120th day in G+ media, where on 120th day The cells in medium with added glucose varied in morphology (Fig 8 A, B). The size of the cells increased from 0.55 ± 0.10 µm to 5.0 ± 1 µm. The shape of the cells varied from circular to oval on the initial day to long slender rods at the

end of 120 d and produces EPS around cells which directly indicates higher mobilization of metal in media (Fig. 8A, B). The cells in 'G+' medium directly attached/sitting to basalt surfaces on 0 day but on 120th day they produced EPS on rock surfaces (Fig. 8 C and D). In contrast, in 'G-' medium, there was no EPS production, variation in cell size or change in morphology when compared 0 day with 120th day incubation. Compositional analyses of basalt fragments after incubation showed the loss of Mn and Cu and gain of Na and Mg in the experimental flasks (Table 2).

Table 2. EDS point analysis of Mn oxide coatings on basalt. Element (Wt %) Random EDS points

	Before incubation		After incubation	
Element	1	2	3	4
Na	1.41	2.25	3.13	3.74
Mg	1.31	2.35	2.19	4.93
Al	0.35	0.85	1.75	2.57
Si	3.81	3.33	11.69	8.14
K	0.61	0.95	1.33	0.42
Ca	0.98	1.01	1.18	2.06
Mn	17.78	28.83	4.96	16.44
Fe	12.41	4.42	14.13	10.82
Cu	0.87	1.78	0.77	0.44
Zn	0.64	1.30	0.70	0.39
Oxygen	62.55	52.93	58.44	50.06

On the same time, the basalt rock fragments from the controls appeared unchanged and seemed similar in texture and composition to the natural samples (Fig. 9). These observations strongly suggest the bacterial participation in the mobilization of Mn.

Characterization and identification of bacterial isolate:

Bacterial isolated R6 were Gram positive rods. They showed positive results for some of the extracellular enzymes such as catalase, oxidase, amylase, caseinase, lipase and cellulose. The strain also showed oxidative fermentation and motility. It was later identified as *Imtechella Halotolerans* with 92.6% similarity and uploaded the sequence (accession number: LK934699) to EMBL database.

DISCUSSION

The deep-sea hydrothermal plumes at CR (Quadfasel et al., 1997; Ray et al., 2012) wield an influence on the water column characteristics and support unique kind of microbial life. These geochemical characteristics of water column and deposition of plume on sea surfaces offer encouraging conditions for the growth and activity of metal immobilizing/mobilizing microorganisms. In the present study Mn concentration in basalt reported around 6.2 to 26.96%, which are similar to the previous reports (25.5 to 65.2%) from the Galapagos spreading axis (Moore and Vogt, 1976). In present samples Mn concentration observed lower than Galapagos due to more distance of the sampling point from the potential volcanic sources.

Also, Fernandes et al 2005 and Sujith et al 2014 observed a large number of Mn oxidizing bacteria allied with the Mn-oxide coatings on basalt rock surfaces. The culturability of Mn oxidizing and general heterotrophic bacteria from these samples showed equal kind of physical characters except for the colour on medium amended and unamended with Mn.

R6 isolated from same area on Mn amended media plate. Same Mn oxidizing bacteria R6 used in present study for Mn mobilization in

Table 3. Phenotypic characteristics of culturable bacteria R6 associated with basalt.

Isolates	Gram ±	KOH	Motility	OF Test	Catalase	Oxidase	Amylase	Dnase	Protease	Lipase Cellulase
R6	+	NT	Motile	F	+	+	+	-	+	+ +

Isolate numbers R6 represent Mn oxidizing bacteria. OF = oxidation fermentation, O= oxidative, F= fermentative. NT= no thread formation and T = thread formation in KOH test.

laboratory condition to solve the purpose of Mn biomining from deep sea ridge basalts. The mobilization of Mn was shown on Mn-oxide plate when R6 reduces Mn oxide and developed zone around bacterial colony. So, this observations suggest that mobilization of Mn from the basalt surfaces could be mediated by bacteria that could either oxidize Mn(II) or reduce Mn-oxide, depending on the ambient conditions. The present results follows the report of Bromfield and David (1976) on Mn mobilization from Mn-oxide by *Arthrobacter* sp. that had the ability to either mobilize or immobilize Mn, depending on conditions. Furthermore, Thamdrup (2000) reported different strains of Mn and Fe oxidizing *Bacillus* sp. and Mn-oxidizing *Bacillus* SG1 who were able to reduce or oxidized metal under anaerobic conditions. Mn-oxides reduction is reported by different bacterial genera includes *Bacillus*, *Deferribacter*, *Thermoanaerobacter*, *Fervidobacterium*, *Desulfovibrio*, *Shewanella*, *Achromobacter*, *Enterobacter Salmonella* and *Halotolerance* sp (Ehrlich, 1980; Pak et al., 2002; Das et al., 2011;Sujith et al 2014).The bacterial communities present in basaltic environment may transfer to high affinity transport systems (Geesey and Morita, 1979) as they not contains any energy reserves (lipids and poly-β hydroxyl butyrate) essential for the life maintenance (Morita, 1988). So, nutritional requirements of such microbes are depend on exoenzymes production or depend upon syntrophism for their energy needs. In above study bacterial isolate R6 expressed multiple exoenzymes with different growth substrates and also oxidized Mn. It directly indicates R6 having high efficiency to adapt nutrient poor environment and meeting the metabolic requirements. Our results favoring the work of Lorenz et al. (2006) and De Souza et al. (2006) towards the bacterial tolerance to the heavy metals and their ability to express multiple enzymes. Novitsky and Morita, 1978 suggested that in low nutrient availability, bacteria may lose the ability of multiplication but energy-yielding mechanism tends to remain intact. Our results followed same pattern as G- shows higher onset growth than G+ in media may be due to the laboratory conditions mimicked the natural conditions to the some extent more in terms of temperature and ambient organic carbon concentration (glucose). In contrast, organisms in 'G+' media may required adaptation period in a non-energized state. While in presence of glucose R6 became chemotactially more active than the non-energized cells present in media without glucose this could be directly responsible for higher mobilization of Mn (Torrella and Morita, 1981). Glucose oxidation by R6 in presence of basalt leads to the reduction of Mn-oxide which directly supports Roh et al. (2002) theory of glucose utilization by heterotrophic bacteria as an electron donor for the reduction of Mn oxide proposed by Madgwick (1987) and Baglin et al. (1992) with reference to the solubilisation of Mn from ores. Although the pH of seawater ranged from 7.5 to 8.4 and pH decrease may occur during bacterial growth under experimental conditions. The pH drop is strongly influenced by bacterial growth (Li et al., 2006) and influence the release of metal cations by promoting an increase in the solubility of metal oxides (Burkhardt et al., 2011). In the present study the pH decreased from 8.1 to 7.2 in the 'G+' and from 7.9 to 7.2 in the 'G-' in media. Our results supports Rusin and Ehrlich (1995) on Mn-oxide reduction by bacteria in the pH range of 6 to 8. Here R6 shows significant reduction associated with basalt and participate in the cycling of Mn vary in their tolerance to different pH levels and metal concentrations. Bacteria also affects or mediates redox cycling of Mn under optimal Eh conditions (Webb et al., 2005) because of the general relationship between pH and Eh (Pareuil et al., 2008). In bacterial growth and metabolic activity either pH of surroundings either increases or decreases. Mobilization of metallic elements directly influenced by increase in H+ activity (drop in pH) which leads to Eh decreases. (DeLaune and Reddy, 2005; Pareuil et al., 2008).

In our study, the Eh varied between +117 to -116 mV in media with added glucose and +137 to -118 mV in media without added glucose. Our results support Mn-oxide reduction by bacteria in the optimal pH and Eh range. The Eh values indirectly indicates less presene amount of

reduced Mn and/or Fe compounds and more of oxidized or bounds state compounds in basalt. Heavy metal stress, nutrient deprivation and excess nutrient availability directly influences morphological variation in bacteria (Shehata and Marr, 1971; Amy and Morita, 1983; Antony et al., 2011). Sujith et al., 2010 and Antony et al., 2011 reported that low metal concentrations i.e., <10 μM stimulate bacterial growth but higher concentrations may leads to toxicity. R6 shows the similar results, reported by Sujith et al., 2010 and Antony et al., 2011 that Some bacteria decreases their cell surface area to adapt to higher metal concentrations for instance and some increases their cell size by forming cell aggregates.

In this study R6 bacteria was very tiny coccoidal in initial day incubation became long slender rods in medium with added glucose at the end of incubation. There was no change in cells size and shape in medium without added glucose. These observations supports Shehata and Marr (1971) an increase in bacterial size and numbers during growth in the presence of 0.1% glucose. EPS secretion and association with the bacterial cells in medium indicates metal stress tolerance and promote Mn dissolution. Similar results were shown by R6 in SEM analysis. R6 bacteria stand with heavy metal stress in the presence of medium amended with glucose. The secretion of EPS or slime layers helps bacteria in attaching to the surfaces, dissolution of minerals and in the alleviating the toxic effect of metals (Geesey and Jang, 1989; Vandevivere and Kirchman, 1993).

Following incubation, EDS analysis of the basalt surfaces explains the elemental loss and gain in addition to colonization by R6 bacteria in the experiment relative to the controls. It showed the direct participation of R6 bacteria in the mobilization of Mn from the basalt surfaces. The mineralogy of the Mn-oxide coatings on basalt showed characteristic peaks for Mn minerals todorokite and birnessite apart from background peak for halite. The present results supports the reported mineralogy of Mn-oxides from the mid-ocean ridge systems (Rao and Pattan, 1989 and Gitanjali B, 2015).

The identification of culturable bacteria R6 showed taxonomic affinities to *Imtechela Halotolerance.* The present study suggest that the Mn(II)-oxidizing bacteria R6 from the CR actively participate in promoting Mn-accretion at ambient concentrations of organic carbon. It also describes Mn-mobilization under conditions of organic carbon enrichment. This could be biogeochemically significant because of the redox speciation of Mn controls the fate of several other associated elements in the ridge ecosystem by consuming or releasing electrons or protons. Experiments conducted under microaerophilic and anaerobic conditions would throw more light on particle associated bacterial contribution from the water column above the redox changes in Mn chemistry in ridge ecosystems.

CONCLUSION

Bioreduction/Mobilization of Mn natural oxide using the R6 bacteria *Halotolerance sp* was dependant on the pH, initial Mn natural oxide concentration and metabolic activity of the organism. The mobilization rate of Mn was maximum at the end of 120 d incubation in 'G+' 27985.91 μg g^{-1} d^{-1} and 4797.37 μg g^{-1} d^{-1} in 'G-' media. Results indicated that the bioreduction/mobilization of Mn is 43 times more in presence of Glucose at pH 6.01. Mn found to important role in Bacteria metabolic activity shows in SEM results and same time bacteria develops some crystal particles at end of experiment which would explain in further study. So, R6 can be widely use for bio mining without any chemical pollution to Environment.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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