Deep-Sea Research II 118 (2015) 197–208

Contents lists available at ScienceDirect

Deep-Sea Research II

journal homepage: www.elsevier.com/locate/dsr2

Response of polar front phytoplankton and bacterial community to micronutrient amendments

Anand Jain [a,d], Ram Murti Meena [a], Ravidas K Naik [c], Jasmine Gomes [a], Mandar Bandekar [a], Mithila Bhat [b], Analia Mesquita [b], Nagappa Ramaiah [a,*]

[a] Microbial Ecology Laboratory, Biological Oceanography Division, Dona Paula, Goa 403004, India
[b] Chemcial Oceanography Division, CSIR-National Institute of Oceanography, Dona Paula, Goa 403004, India
[c] Southern Ocean Group, National Center for Antarctic Ocean Research, Sada Headland, Vasco-da-Gama, Goa, India
[d] Arctic Group, National Center for Antarctic Ocean Research, Sada Headland, Vasco-da-Gama, Goa, India

ARTICLE INFO

Available online 31 January 2015

Keywords:
Southern ocean
Polar front
Micronutrients
Microcosm
Phytoplankton
HPLC
Bacterial community
DGGE

ABSTRACT

In a shipboard microcosm experiment we manipulated cobalt (Co), copper (Cu), iron (Fe), and Fe+Co availability in surface water samples collected during austral summer of 2013 from a Polar front (PF) location in the Indian Sector of Southern Ocean, to examine the responses of phytoplankton as well as bacterial community (BC) to these micronutrient amendments. Total chlorophyll *a* (Chl *a*) concentration increased significantly ($P < 0.01$) in all micronutrient-enriched microcosms (MEM), with the incubation period unlike in the control microcosms (CM). Bacterial abundance in Co, Fe and Fe+Co-enriched microcosms increased 1.5 fold within 5 days of incubation, unlike in other microcosms. Despite significant increase in Chl *a*, the DGGE banding pattern reflects minor differences in the BC. However, there were significant differences in relative abundance (i.e band intensity) of two most responsive phylotypes identified as *Rhodospirillales* and *Rosebacter* sp of *Alphaproteobacteria* group between MEM and CM. Strong correlation was not discernable between Chl *a* concentration and bacterial abundance as well as BC structure. Apparently autotrophic processes in this region are not only limited by the availability of Fe, but also Co and Cu.

1. Introduction

A third of the 108 elements are known to have biological functions (Brantley et al., 2001). Of these elements, the trace metals/micronutrients, such as Mn, Fe, Co, Ni, Cu, Zn, and Cd are found specifically as cofactors or as part of cofactors in enzymes and as structural entities in various proteins (Butler, 1998). In the marine ecosystem, trace metals are required in various processes such as photosynthesis (Morel et al., 1991), carbon fixation (Reinfelder et al., 2000), and in various biogeochemical processes (Morel et al., 2003, Morel and Price, 2003). However, these trace metals/micronutrients are present in very low concentrations in the open ocean (Ellwood, 2004, 2008; Ellwood et al., 2005; Croot et al., 2011; Hassler et al., 2012). They readily react with organic compounds and organic ligands (Ellwood, 2004, 2008; Ellwood et al., 2005; Lohan et al., 2005). As a result their bioavailability to sustain phytoplankton is reduced. The effect of trace metal availability on primary productivity/phytoplankton growth, community composition and carbon sequestration has been reported earlier (Morel et al., 1991; Morel et al., 1994; Sunda and Huntsman, 1995; Coale et al., 1996; Boyd et al., 2000; Buitenhuis et al., 2003; Saito and Goepfert, 2008; Saito et al., 2008). However, the impact of these dynamics on bacterial community (BC) structure is not that well understood.

Heterotrophic bacteria are critical part of 'biological carbon pump' and influence the efficiency of carbon export via mineralization of organic matter back to inorganic phase and/or conversion of organic matter to biomass (Robinson and Ramaiah, 2011). It has been shown that trace metal limitation (particularly Fe) leads to the lower electron transport chain (ETC) activity and reduce carbon growth efficiency in marine bacteria (Tortell et al., 1996). As a result, bacteria mineralize a large proportion of ingested carbon at the expense of biomass production (Kirchman et al., 2003). This retards the carbon export to deeper depth from the surface of the ocean. The high surface area to volume ratio of bacteria and their ability to produce the organic ligands make them well suited to effectively scavenge trace elements (Azam et al., 1983; Granger and Price, 1999). However, despite of all these adaptations information on growth and activity of bacteria in Southern Ocean regions are limited. Understanding factors controlling bacterial growth, activity and community composition can provide

* Correspondence to: Biological Oceanography Division, CSIR-National Institute of Oceanography, Dona Paula, Goa, 403004. Tel.: +91 832 2450 515; fax: +91 832 2450 606.
E-mail address: ramaiah@nio.org (N. Ramaiah).

http://dx.doi.org/10.1016/j.dsr2.2015.01.009
0967-0645/

insights on the degree to which they can mediate organic carbon fluxes in the Southern Ocean.

Earlier studies reported iron limitation (Pakulski et al., 1996), dissolved organic matter (DOM)/dissolved organic carbon (DOC) limitation (Hall and Safi, 2001; Oliver et al., 2004) and co-limitation by iron and DOM together (Church et al., 2000) as limiting factors of bacterial growth and activity in Southern Ocean regions. Generally, most of these studies focused on bacterial abundance and activity. However, only fewer studies reported response of BC to iron (Hutchins et al., 2001, Thiele et al., 2012) and organic matter (Simon et al., 2012) addition in Southern Ocean regions. Particularly, the effect of other micronutrients Co and Cu, which share several properties with Fe in limiting the growth of phytoplankton and/or in mediating their community structure (Morel et al., 1994; Sunda and Huntsman, 1995; Buitenhuis et al., 2003; Saito and Goepfert, 2008; Saito et al., 2008), on BC in the Indian sector of the Southern Ocean, remains to be deciphered. In this study we manipulated cobalt (Co), copper (Cu), and iron (Fe) availability in the samples collected from polar front of Indian sector of the Southern Ocean, in order to examine their impact on total chlorophyll *a* (Chl *a*), phytoplankton community, bacterial abundance as well as community structure. Specifically, we intended to study the coupling between phytoplankton growth and BC structure under these micronutrient amendments.

2. Materials and methods

2.1. Microcosm experimental setup

A microcosm experiment was conducted in a Polar front (PF) location in the Indian Sector of the Southern Ocean [latitude: 56°35′0.235S; longitude: 57°40′0.342E] (Fig. 1), on-board ORV Sagar Nidhi at 10.00 AM on February 5, 2013. For this experiment, 120 l of unfiltered surface seawater was collected using an acid cleaned polyethylene bucket. In the present study unfiltered seawater samples were used to perform the microcosm experiments in order to avoid diversion from natural conditions as has been suggested by Banse (1991), Bumaet al. (1991), and Scharek et al. (1997). The seawater was distributed (4.5 L per flask) to pre-cleaned flasks of 5 L capacity. Prior to dispensing seawater into them, the flasks were sequentially cleaned with detergent, 50% v/v nitric acid (ACS grade, Sigma-Aldrich, India), 10% v/v hydrochloric acid (ACS grade, Sigma-Aldrich, India). The flasks were allowed to retain 10% hydrochloric acid rinse for 1 week before being rinsed and filled with ultrapure water (Type-I grade) one day before the experiment. This ultrapure water was discarded and flasks were finally rinsed with the sample seawater. Trace metals as chloride salts obtained from Sigma-Aldrich, India were dissolved in ACS grade 0.01 N HCl, pH∼1.0. After filling with sample seawater, 1 ml of concentrated trace metal solutions viz, Co, Cu, Fe, and Fe+Co were added separately to the flasks so as to attain a final concentration of 10 nM L^{-1}. The concentration of trace metals for the amendment experiment was chosen based on published literature. The reported range of trace metals in microcosm experiments range from 0.5 to 10 nM (Coale, 1991, Scharek et al., 1997, Church et al., 2000, Kirchman et al., 2000, Olson et al., 2000, Hutchins et al., 2001, 2002, Becquevort et al., 2007, Eldridge et al., 2007, Ramaiah et al., submitted). However, in the bottle incubation experiments, part of trace metal added become complexed by the dissolved organic matter (Ellwood, 2004, 2008; Ellwood et al., 2005; Lohan et al., 2005) and a portion of added trace metals will probably be adsorbed to the walls of the bottle, and become unavailable for phytoplankton (Sunda, 1988, Coale, 1991, Scharek et al., 1997). To ensure sufficient concentration of bio-available/bio-assessable trace metals during the 15 day long incubation experiment 10 nM concentration of trace metals was added.

All the flasks were incubated in the shipboard refrigerated cold room at 4 C° ± 1. To avoid any trace metal contamination the inner walls of the cold room were covered with the thin plastic sheets prior to the experiment. A total of 15 flasks including 12 treatments (three flasks per treatment or micronutrient amendment) and 3 controls were exposed to a light intensity of 150 μmol photons m^{-2} s^{-1} (under cool fluorescent light) under 16:8 h light:dark cycle. Microcosms were maintained at light intensities of 150–160 μmol photons m^{-2} s^{-1} to mimic the in-situ PAR∼170 μmol photons m^{-2} s^{-1} (Gandhi et al., 2012). From these a set of five flasks (one control and four treatments) were removed for collecting samples for total Chl *a*, phytoplankton pigments, bacterial abundance, and bacterial community analysis at 5 days interval, until 15th day. To

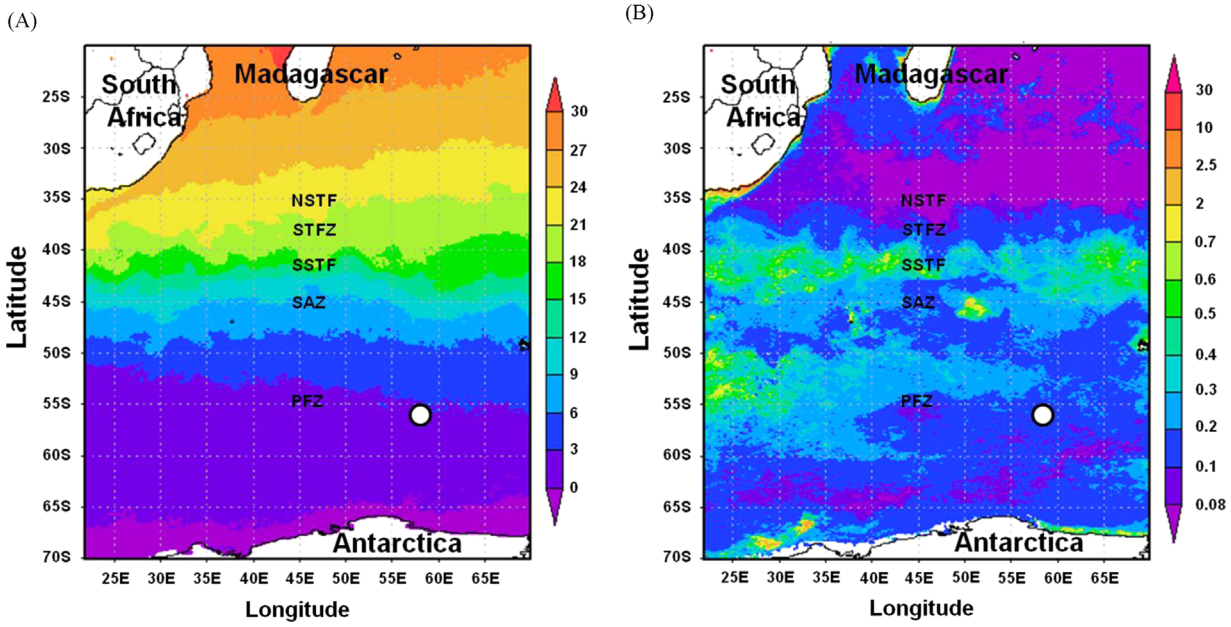

Fig. 1. Monthly mean (A) sea surface temperature (SST) and (B) chlorophyll *a* (Chl *a*) concentration obtained from MODIS-aqua for the period of Jan to March 2013 is plotted with the sampling location (shown as white circle). The fronts and frontal zones at 45°E longitude are marked from Anilkumar et al. (2005, 2006, 2007). NSTF, North subtropical front; STFZ, Subtropical frontal zone; SSTF, South subtropical front; SAZ, Sub-antractic zone; PFZ, Polar frontal zone.