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Full Length Research Paper

Metaproteomic evaluation on the microbial community functions in a mesoscale cyclonic eddy perturbation

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Eddy pumping is a process by which mesoscale eddies induce isopycnal displacements that lift nutrient-replete waters into the euphotic zone, driving a set of biogeochemical processes in the ocean. To investigate the potential effect of physical processes on the microbial community functions, the microbial community metaproteomic profiles were determined in a cold-core cyclonic eddy in the South China Sea (SCS). The significant differences of metaproteomic patterns accurately predicted their distinct functional responses to environmental changes caused by the mesoscale cyclonic eddy perturbations. From the protein gel profiles, 11 proteins were successfully identified by MALDI TOF MS/MS, including nitrogen regulatory proteins, ribosomal proteins and substrate transport proteins, etc, which implied the metabolic responses of the microbial populations to the nutrients change within the cyclonic eddies. These results highlight the potential of metaproteomics for studying complex microbial consortium in the ocean and contributed to our understanding of the biological consequences of a mesoscale cyclonic eddy in the SCS.

Key words: Cyclonic eddy, metaproteomics, microbial community.

INTRODUCTION

The South China Sea (SCS) with its deep basin is one of the largest marginal seas in the tropical Pacific Ocean. It is characterized by the relatively frequent passage of eddies (Hwang and Chen, 2000; Wang et al., 2003), which usually induced perturbations of biogeochemical processes including recycling of nutrients within the surface sunlit waters or physical transport of nutrients from nutrient-rich deep waters, thus influence the productivity and community species composition of the autotrophic organisms, zooplankton grazing, and “biological pump” (Benitez-Nelson et al., 2007; Benitez-Nelson and McGillicuddy, 2008; McGillicuddy et al., 2007).

During these processes, it was reported that microbial communities usually have remarkable dynamic responses in terms of their growth rates, productivity, and community composition, etc. For instance, Hanson et al. (1986) reported that the microbial growth rates in the mixed surface waters of eddy were 10 times higher than in the adjacent waters of the northwestern Sargasso Sea. Also, Ewart et al. (2008) revealed that there was systematic variability in bacterioplankton dynamics in two upwelling eddies in the Sargasso Sea. Recently, Baltar et al. (2010) systematically investigated the effects of mesoscale eddies on prokaryotic assemblage structure and activities in the permanent eddy-field downstream the Canary Islands, and found apparent variations in prokaryotic abundance, heterotrophic activity, single-cell activity (such as nucleic acid content, proportion of live cells, and fraction of cells actively incorporating leucine), as well as the bacterial and archaeal community structure. However, so far little is known regarding how microbial metabolic functions respond in mesoscale eddies.

It is well known that in the postgenomic era, the large-scale study of proteins expressed by indigenous microbial communities (metaproteomics) is an effective approach for studying functional microbial ecology, providing information to gain insights into the activity of

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the microbial ecosystems (Maron et al., 2007). In this study, with the objective of evaluating the functional responses of microbial community to mesoscale eddy perturbation, a 2D gel based proteomic method coupled with MALDI TOF MS/MS for protein identification were employed to investigate the bacterial community functions in two cold-core cyclonic eddies in the SCS in summer. The results contribute to our knowledge about the functional activities of microbial communities under the influences of the biogeochemical perturbations of a mesoscale cyclonic eddy in the SCS.

MATERIALS AND METHODS

Sample collection

For metaproteomic analysis, four samples were collected from 5 m and the chlorophyll maximum layers at two stations. One was located inside the cold-core cyclonic eddy (CE1 at 111.83°E, 14.25°N), and the other was at the Southeast Asia Time-Series Study station (SEATS at 115.96°E, 18.03°N). Briefly, 100 L of sea water was pre-filtered through 3 µm-pore-size polycarbonate filters (Whatman) to remove large particles and eukaryotes. Microbial cells in the filtrate were concentrated to a final volume of 200 ml using a tangential-flow ultrafiltration system (30000 MW cutoff) as described elsewhere (Chen et al., 1996). The concentrated samples were flash-frozen in liquid nitrogen and stored at -80°C until protein extraction.

Protein extraction and purification

Microbial cells in the retentate were pelleted by centrifugation at 13000 × g, 4°C for 10 min. The collected cells were rinsed with TS washing buffer (Tris-HCl 10 mM, sucrose 250 mM, pH 7.6) and re-suspended with 0.3 ml of extraction buffer. The extraction buffer was prepared according to the previously described method (Kan et al., 2005). It consisted of 0.01 M Tris-HCl, pH 7.4, 1 mM EDTA, 7 M urea and 2 M thiourea, 10% (v/v) glycerol, 2% CHAPS, 0.2% ampholytes, 0.002 M Tributyl phosphine (TBP), DNase I (0.1 mg ml^{-1}), RNase A (0.025 mg ml^{-1}) and proteinase inhibitor cocktail (CalBiochem). TBP, DNase I, RNase A and proteinase inhibitor cocktail were freshly added to the buffer prior to applying to samples. Large cellular debris was removed by centrifugation (10000 × g, 4°C for 5 min). Protein concentration of the supernatant was determined using the Quick Start Bradford Protein Assay kit 1 (Bio-Rad) following the manufacturer's instructions.

Isoelectric focusing (IEF) and SDS-PAGE

The first dimension separation of proteins was carried out in the immobilized pH gradient (IPG) strips (18 cm, pH 3 to 10) on an IPGphore system (GE, Healthcare). Each two-dimensional gel electrophoresis (2D-PAGE) was conducted using 200 µg of total protein. The IEF program was: active rehydration at 50 V for 13 h, 250 V for 15 min followed with a linear ramp to 1000 V for 1 h, and then from 1000 to 8000 V for 2 h, and the last step, at 8000 V for a total 60000 Vh with a rapid ramp. After the first dimension, the IEF strips were equilibrated in Equilibration Buffer I and II (Bio-Rad) for 15 min each. The second dimension of 2D-PAGE was performed using 12.5% SDS-polyacrylamide gels in the Protean II XL (Bio-Rad) system at 150 V for 30 min, then at 200 V for 5 to 6 h until the bromophenol blue reached the bottom of the gels. The gels were stained with SYPRO Ruby (Bio-Rad) after electrophoresis and scanned using the Gene Genius Bio Imaging System (Syngene).

Metaproteome image analysis

Images were analyzed and quantitatively compared using ImageMaster 2D Platinum 5.0 software (GE Healthcare). Gel images were compared in multiple gel modes by measuring the total density in gels for spot quantification. All gels were subjected to the same spot detection parameters followed by automated matching. Then pairwise comparisons of gels were inspected and matches edited manually to eliminate poor quality or low intensity matches. When automatic matching failed, the number of matched and unmatched spots was estimated by manual examination of overlaid 2D SDS-PAGE images.

Protein identification by mass spectrometry (MS)

Interesting protein spots were manually excised from gels using Pasteur pipettes and digested as described by Shevchenko et al. (1996). Tryptic peptides were analyzed via MALDI-TOF MS and MS/MS. Briefly, after digestion, the extracted peptides were dried under the protection of N_2. For MALDI-TOF MS, the peptides were eluted onto the target with 0.7 µl of matrix solution (α-cyano-4-hydroxy-cinnamic acid in 0.1% trifluoroacetic acid, 50% acetonitrile). Samples were allowed to air dry before being inserted into the mass spectrometer (ABI 4700 Proteomics Analyzer [Applied Biosystems]). Positive ion mass spectra were recorded on a home-built linear time-of-flight mass spectrometer using 20 kV of total acceleration energy. Data from MALDI-TOF MS/MS were analyzed using the search software MASCOT (Matrix Science).

RESULTS

Total 200 µg of the extracted protein of each microbial community was analyzed based on 2D-PAGE coupled with mass spectrometry (MS). The 2D-gel images from the surface and the chlorophyll maximum layers of CE1 and SEATS were compared (Figure 1). Image overlays were constructed using ImageMaster 2D Platinum 5.0 software. Replicate metaproteome images from the different samples were similar to one another, sharing 96-98% of all detected spots. The metaproteomic patterns shown in the 2D-gels from the different sites varied significantly from each other. However among them, the microbial metaproteomes from the surface and the chlorophyll maximum layers of SEATS are relatively similar to one another with ~25% of all detected spots in common. While the metaproteomes from the surface and the chlorophyll maximum layers of CE1 only shared about ~10% of detected spots.

To preliminarily evaluate the microbial community functions, 16 interesting protein spots in the metaproteomic gels (Figure 1) were punched out and identified using MALDI-TOF MS/MS and MASCOT. A protein score of more than 69 and at least 2 matched peptides per protein were set as the threshold for positive identification. A large portion (that is, 11 proteins) of the punched protein spots were identified successfully (Table 1). The potential functions of microbial community were

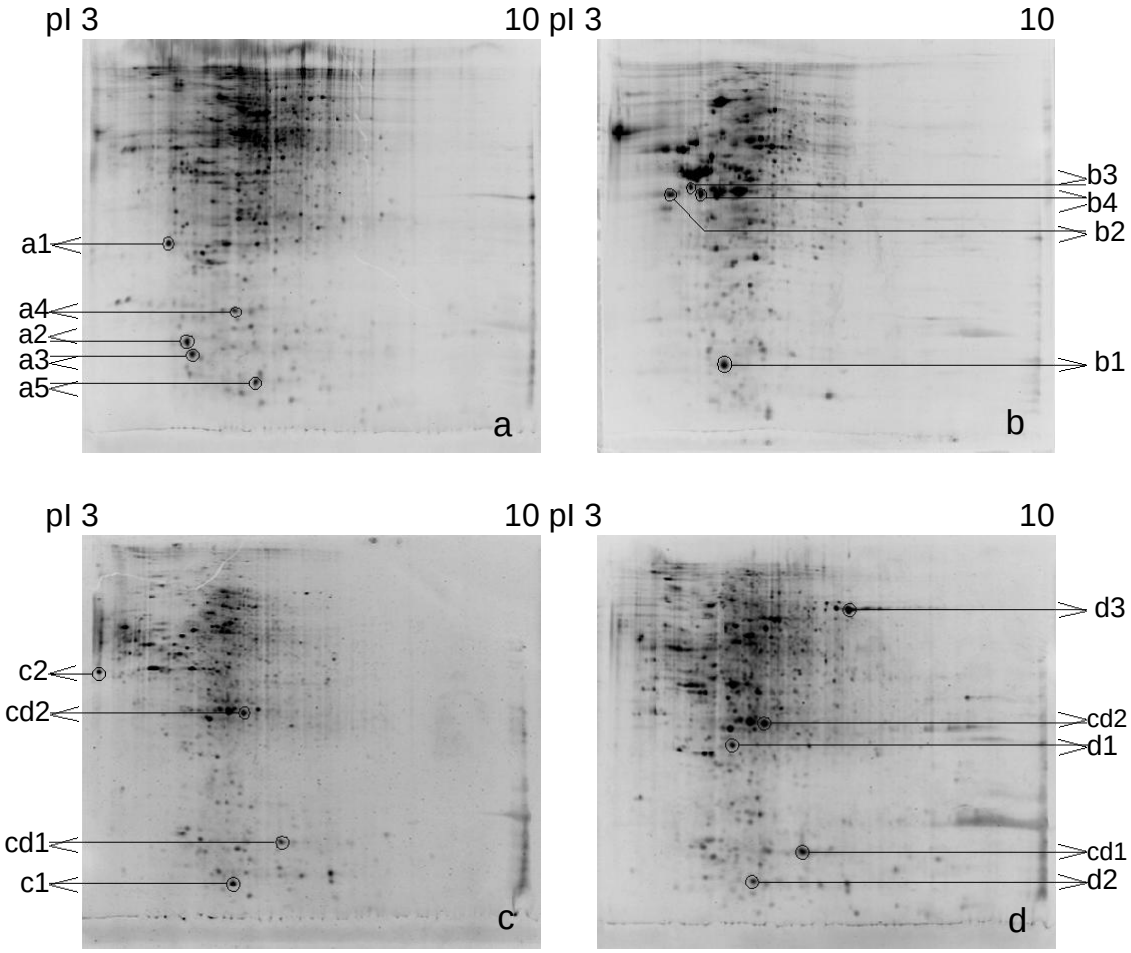

Figure 1. Metaproteomes of microbial communities. (a) the surface water of CE1; (b) the chlorophyll maximum layers of CE1; (c) the surface water of SEATS; (d) the chlorophyll maximum layers of SEATS. Descriptions of stations are as in Figure 1. pI: isoelectric point. Spots circled were punched out for protein identification using MALDI TOF MS/MS. Spots marked as a1-5, b1-4, c1-2 and d1-3 were unique on (a), (b), (c) and (d) gels, respectively. Spots marked as cd1-2 were common on (c) and (d) gels.

discussed thus.

DISCUSSION

Aiming to make a preliminary investigation of the microbial community functions, metaproteomics as a key technology for function analysis of microbiome was carried out in this study. This can often provide a direct measurement of functional gene expression in terms of the presence, relative abundance and modification state of proteins, and link *in situ* functions of microbial community to the challenging environments. We explored the proteome profiles from bacterioplankton communities between 0.2 to 3.0 microns in size by the choice of prefiltration and ultrafiltration cut-off sizes. The 2D-gel based metaproteomic analysis typically resolved hundreds of major proteins from the microbial communities, and provided sufficient resolution and reproducibility to detect protein expression patterns of microbes.

When comparing the protein profiles of the four microbial communities from the surface and the chlorophyll maximum layers of CE1 and SEATS, significant differences were found between the two sites of CE1 and SEATS, and between the surface and the chlorophyll maximum layers at the site CE1 with as little as ~10% of protein spots in common (Figure 1). This might be partially explained by the difference among the population structures of these samples. The protein based analysis revealed that the microbial community functions were influenced significantly by the environmental perturbations caused by the cyclonic eddies.

Previous studies indicated that protein identification is the major challenge for metaproteomics (Kan et al., 2005; Wilmes and Bond, 2006). Here, with the objective of investigating preliminarily the microbial community

Table 1. Identification of proteins from the metaproteomes of sites CE1 and SEATS. Protein No. is shown in Figure 1.

Protein No.	Protein name	Score	No. of matched peptides	Origin
a1	TonB system biopolymer transport component	171	6	*Alteromonas macleodii* 'deep ecotype'
a2	Ribosomal protein L7/L12	336	7	*Alteromonas macleodii* 'deep ecotype'
a3	Hypothetical protein Ccur5_01000144	111	5	*Campylobacter curvus* 525.92
a4	Nucleoside diphosphate kinase	262	7	*Alteromonas macleodii* 'deep ecotype'
a5	Nitrogen regulatory protein P-II	401	15	*Alteromonas macleodii* 'deep ecotype'
b1	50S ribosomal protein L7/L12	114	5	*Mesorhizobium loti* MAFF303099
b2	Immunogenic protein	98	2	*Loktanella vestfoldensis* SKA53
c1	Carboxysome shell protein CsoS1	385	10	*Prochlorococcus marinus* MIT 9312
c2	Outer membrane porin, putative	640	8	*Roseobacter denitrificans* OCh114
d1	Alkyl hydroperoxide reductase	148	7	*Shewanella amazonensis* SB2B
d2	Nitrogen regulatory protein P-II	258	12	*Alteromonas macleodii* 'deep ecotype'

function, we selected 16 interesting protein spots in the metoproteomic gels (Figure 1) for MALDI-TOF MS/MS and MASCOT analysis. About 69% of the selected protein spots were identified successfully. In the surface water of the site CE1, four of five unique proteins were successfully identified as cellular proteins or ribosomal proteins of *Alteromonas macleodii* 'deep ecotype' (Table 1). Notably, nitrogen regulatory protein P-II was abundant in the surface sample of CE1, which belongs to a family of signal transduction adaptor proteins that control the metabolism of nitrogen, such as nitrogen fixation and nitrogen assimilation (Martin and Reinhold-Hurek, 2002). We supposed that the microbial nitrogen metabolism was much active in this environment. Additionally, biopolymer transport protein was also abundant in the surface sample of CE1, which belongs to membrane bound transport proteins essential for ferric ion uptake in bacteria (Wiggerich et al., 1997). These functions of microbial community could link to or explain the rapid increased N:P ratio (up to ~40) and the relatively iron limitation within the cyclonic eddies. At the chlorophyll maximum layer of the site CE1, two of four unique proteins were successfully identified by MALDI TOF MS/MS, of which immunogenic protein is related to transporting substrates, such as amino acids, peptides, sugars and vitamins.

For the two samples of the site SEATS sharing ~25% protein spots, we picked out two proteins in common for identification but failed. However two unique proteins in the surface sample were identified successfully, one of which was carboxysome shell protein CsoS1 from *Prochlorococcus marinus* MIT 9312 responsible for carbon dioxide-concentrating mechanism. Another was outer membrane porin with transporter activity. At the chlorophyll maximum layer of SEATS, nitrogen regulatory protein P-II and the antioxidant protein alkyl hydroperoxide reductase were abundant. We suppose that these functions of microbial community were likely to be related to the nitrate-deoxidizing / hydrogen-oxidizing lithotroph *Paracoccus* dominating the site SEATS.

In this exploratory study, although only a small part of the metaproteome were selected for MS identification, it revealed interesting aspects of the functional gene expression within the microbial habitats of seawater that contains high microbial diversity. The results of our experiment highlight the potential of proteomics for the studies of complex microbial consortium in ocean. With more and more metagenomic sequences being deposited in databases in the future, such as the Genbank and CAMERA databases, the application of metaproteomics will contribute effectively to our understanding of the dynamic impacts of cyclonic eddies on microbial community composition and their functions.

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