Distribution and Phylogenetic Diversity of CbbL Gene Encoding RuBisCo in the Deep-sea Sediments from the South China Sea

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Abstract: RuBisCO is the key enzyme catalyzing the first and major step of carbon fixation in Calvin cycle. With the aim to examine the phylogenetic diversity of RuBisCO genes, cbbL gene was amplified by PCR from three South China Sea deep-sea sample, cloned, and sequenced. A total of 28 OTUs deriving from 236 cbbL clones covered 16 phylotypes which all belong to Proteobacteria. The estimated coverage values showed that more than 85% of bacterial cbbL diversity was captured. Shannon and Simpson indices indicated a high diversity of the total cbbL gene in the SCS deep-sea sediments. In conclusion, microbial RuBisCO genes in the South China Sea display a broad range of phylogenetic diversity. The predominant group in these three deep-sea sediments included Thiobacillus and Thiorhodococcus, which was chemoautotrophic bacteria involving in Calvin–Benson–Bassham cycle. The above results implicate that bacteria with the potential for carbon dioxide fixation and chemoautotrophy oocur in the South China Sea.

1 INTRODUCTION

CO_2 is the major contributor to global warming and reduction of CO_2 input in atmosphere is essential for control global warming. The ocean is recognized as a huge carbon reservoir. Deep-sea sediments are a huge carbon pool, the study of microbiology in deep-sea ecosystem mediating flows of energy in metabolism will help understand the CO_2 fixation capacity of the marine ecosystem (Coffin, 2004). Biologically mediated CO_2 fixation is a major pathway in marine ecosystem. Microbiological auotrophic CO_2 fixation is widely distributed and adapted to many habitats.

Autotrophic CO_2 fixing bacteria do not belong to a specific taxonomic group and occurs in many species. Most chemolithoautotrophic bacteria mediate autotrophic CO_2 fixation via the Calvin–Benson–Bassham cycle (Shively, et al., 1986; Selesi et al., 2005) RuBisCO is the key enzyme catalyzing the first and major step of carbon fixation in the Calvin cycle and exists in multiple natural forms which differ in structure, catalytic property, and O2 sensitivity. As such, RuBisCO form I-encoding cbbL genes have been used as functional markers for molecular ecological studies of CO_2 assimilative autotrophs in aquatic systems (Yuan et al., 2012; Kovaleva et al., 2011).

The South China Sea (SCS), near to the West Pacific “warm pool” is the biggest and deepest sea in China (Dai et al, 2002). SCS may be enriched in CO_2 fixation bacteria. However, there are no report on the cbbL gene diversity from the SCS at the moment.

In this study, the geographical distribution and phylogenetic diversity of cbbL gene in sediments of the South China Sea were determined with the aim to broaden our view on the diversity of different deep-sea habitat.

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2 MATERIAL AND METHODS

2.1 Sample Collection

Deep-sea surface sediments (5 cm lower than the top layer) were collected using a gravity box-coring device from three sampling site in the Nansha area of the South China Sea in the Spring of 2013. Characteristics of three sampling sites were described in Table 1.

Table 1: Sample information.

Sites	Water Depth(m)	pH	eh (mv)	Temperature	Sample describe
NSCA	1743	7.44	172	2.9	Yellow mud
NSCE	1683	7.35	176	3.0	Brown mud
NSCI	963	7.37	152	11.1	Red mud

2.2 DNA Extraction

Total genomic DNA was extracted from 5 g sediments following with the Rapid Soil DNA Isolation Kit (Sangon Biotech, Shanghai, China) and further purified using the QIAquikPCR purification kit according to the manufacturer's directions.

2.3 Amplification, Cloning and Sequencing of CbbL Gene

The purified DNA was used for cbbL gene amplification using primers 595f and 1387r (Hung et al, 2012). Thermo cycling reaction was as followed:95℃ annealing for 5min;35 cycles of 94℃ for 30 s,52℃for 30 s,72℃for 1min;72℃for 10min.The PCR products was cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) and *Escherichia coli* TOP10 competent cells. The inserted cbbL gene was sequenced by a commercial company (Takara, Dalian).

2.4 Phylogenetic Analysis

Preliminary analysis of the sequences was performed using BLAST (http://www.ncbi.nlm.nih.gov/blast/). The nucleotide and inferred amino acid sequences were aligned with sequences from GenBank using CLUSTAL W (Thompson et al., 1994). The aligned sequence with more than 97% similarity was defined as one Operational Taxonomic Unit (OTU). Phylogenetic tree of bacteria harbouring cbbL gene was constructed using neighbour-joining algorithm within the MEGA 5.0 software. Alpha-diversity indices, including Shannon, and Simpson values, were subsequently calculated by Mothur.

3 RESULTS

3.1 Diversity Analysis of Cbbl Clone Libraries

Bacterial cbbL sequences were obtained from all three samples (NSCA, NSCE, NSCI). A total of 236 clones were achieved from three cbbL clone libraries constructed from the SCS deep-sea sediments. When analyzed using a 97% sequence similarity cut off, the 236 total cbbL sequences formed 28 OTUs. Rarefaction analysis of the clone libraries showed that the species accumulation curves were asymptotic for all three libraries (Figure 1), indicating a good coverage of the total cbbL gene diversity in the SCS deep-sea sediments. The estimated coverage values showed that more than 85% of bacterial cbbL diversity was captured (Table 2). This result indicates the capacity of cbbL clone libraries is large enough for diversity analysis. Shannon and Simpson indices were calculated to evaluate the evenness and diversity of the cbbL at each site. Shannon-Wiener index of cbbL clone libraries from high to low is NSCE, NSCI and NSCA respectively, suggesting the NSCE site sediments is in the highest bacterial diversity among the three sampling sites.

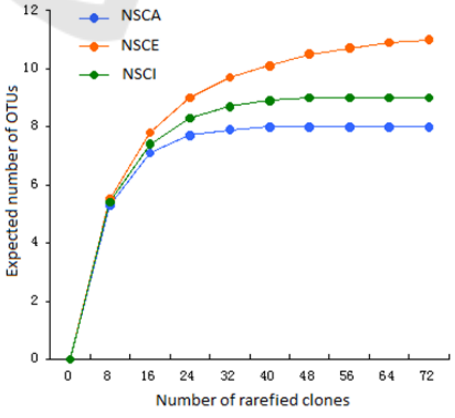

Figure 1: Rarefaction curves for the cbbL.

Table 2: Diversity indices for the cbbL clone libraries.

Clone library	No. of clones	No. of OTUs	[a]Coverage (%)	[b]Shannon-Wiener index	[c]Simpson index
NACS	76	8	89.4	2.03	0.86
NACE	80	11	86.2	2.2	0.88
NSCI	80	9	88.7	2.1	0.87

[a]Percentage of coverage is the percentage of observed number of OTUs divided by the Chao1 estimated value
[b]For the Shannon diversity index, a higher number represents greater diversity
[c]For the Simpson diversity index, a higher number represents greater diversity

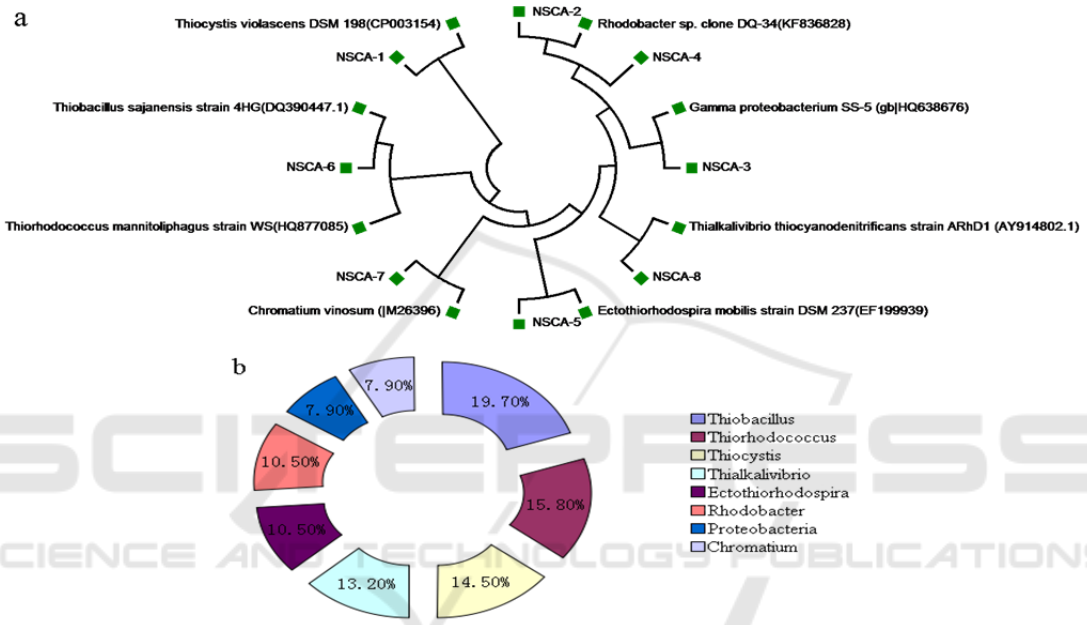

Figure 2: Phylogenetic analysis of bacteria harbouring cbbL gene derived from sediments of NSCA site.(a) Phylogenetic tree based on the cbbL (translated amino acids) sequences;(b)Distribution of cbbL gene in deep-sea sediments.

3.2 Phylogenetic Analysis of Cbbl Genotypes

The expected size fragment was amplified with DNA extracted from three sediment samples and preliminary analysis of sequence yielded positive results with the cbbL gene. A total of 28 OTUs from three cbbL clone libraries covered 16 phylotypes: *Thiobacillus, Thiorhodococcus, Thiomonas, Halothiobacillus, Halorhodospira halochloris, Acidithiobacillus, Ectothiorhodospira, Thiobacillus denitrificans, Nitrosomonas, Hydrogenophaga, Thialkalivibrio denitrificans, Thiocys, Thialkalivibrio, Thiobacillus denitrificans, Chromatium* and *Rhodobacter*. It may be concluded that all of the cbbL sequences detected in Deep-sea sediments belong to the Proteobacteria. The phylogenetic analysis results were matching with the above diversity indices, indicating the diversity of the cbbL gene in the South China Sea.

The bacterial communities harbouring cbbL gene derived from NSCA site were clustered into three classes: α-Proteobacteria, β-Proteobacteria and γ-Proteobacteria; 8 genera: *Thiobacillus, Thiorhodococcus, Thiocystis, Thialkalivibrio, Ectothiorhodospira, Proteobacteria, Chromatium* and *Rhodobacter* (Figure 2). The predominant group in NSCA site deep-sea sediments included *Thiobacillus, Thiorhodococcus, Thiocystis, Thialkalivibrio,* which was chemoautotrophic bacteria involving in Calvin–Benson–Bassham cycle.

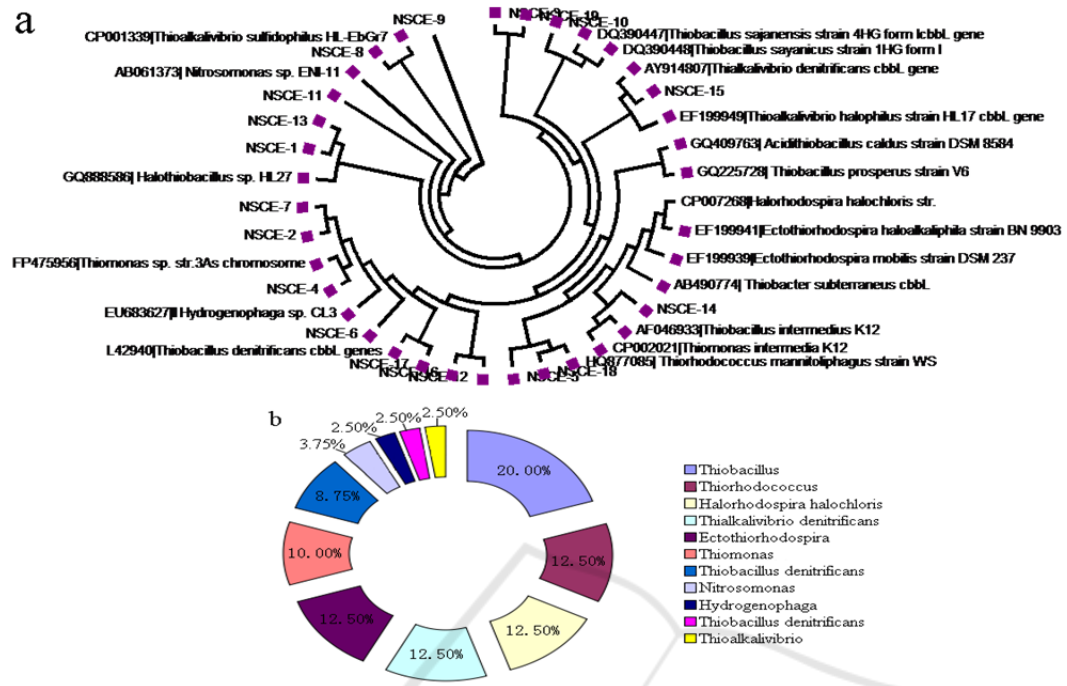

Figure 3: Phylogenetic analysis of bacteria harbouring cbbL gene derived from sediments of NSCE site.(a) Phylogenetic tree based on the cbbL (translated amino acids) sequences;(b)Distribution of cbbL gene in deep-sea sediments.

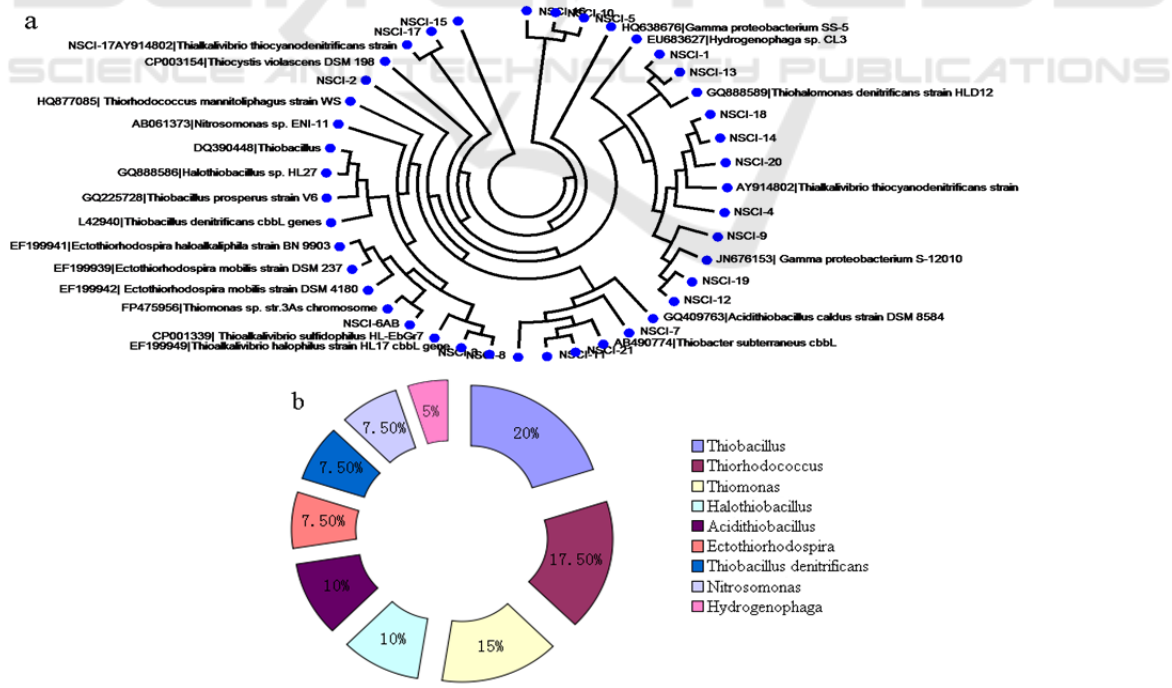

Figure 4: Phylogenetic analysis of bacteria harbouring cbbL gene derived from sediments of NSCI site.(a) Phylogenetic tree based on the cbbL (translated amino acids) sequences;(b)Distribution of cbbL gene in deep-sea sediments.

The bacterial communities harbouring cbbL gene derived from NSCE site were clustered into two classes:β-Proteobacteria and γ-Proteobacteria; 11 genera: *Thiobacillus, Thiorhodococcus, Halorhodospira halochloris, Thialkalivibrio denitrificans, Ectothiorhodospira, Thiomonas, Thiobacillus denitrificans, Nitrosomonas, Hydrogenophaga, Thiobacillus denitrificans and Thioalkalivibrio* (Figure 3). The major bacterial groups in the NSCE site sediments was found to include *Thiobacillus, Thiorhodococcus, Halorhodospira halochloris, Thialkalivibrio denitrificans, Ectothiorhodospira,* also with low abundant of *Nitrosomonas, Hydrogenophaga, Thiobacillus denitrificans* and *Thioalkalivibrio*.

In the phylogenetic tree constructed from the phylotypes of NSCE clone libraries, eleven OTUs could be assigned to three classes: β-Proteobacteria, γ-Proteobacteria and Acidithiobacillia; 9 genera: *Thiobacillus, Thiorhodococcus, Thiomonas, Halothiobacillus, Acidithiobacillus, Ectothiorhodospira, Thiobacillus denitrificans, Nitrosomonas* and *Hydrogenophaga* (Fig. 4). *Thiobacillus* was the most dominant group and accounted for 20% of in NSCI site deep-sea sediments. Other predominant genera in NSCA site deep-sea sediments included *Thiorhodococcus* and *Thiomonas*, which were also chemoautotrophic bacteria involving in Calvin–Benson–Bassham cycle.

4 DISCUSSIONS

The RuBisCO gene were detectable in the SCS deep-sea sediments and the general richness of the cbbL gene was relatively high (from 0.11to 0.14 OTU per clone). Similar results were reported from other habitats. The richness of cbbL genes Giri et al. detected (0.12 OTU per clone) in Mono Lake was comparable to the richness we observed, despite the differences in habitat diversity (Giri et al., 2004). Elsaied et al. identified the richness of cbbL genes (0.10 OTU per clone) covering a range of habitats associated with a hydrothermal vent site, including sediment, overlying water, and as symbionts (Elsaied and Naganuma, 2001). However, RuBisCO genes with low richness were also observed in some extreme habitats such as the deep-sea hydrothermal vents, volcanic deposits and deep hypersaline anoxic basin (Elsaied and Naganuma, 2001; Nanba et al, 2004; Elsaied et al, 2007; Wielen, 2006). Therefore, the diversity of the cbbL gene may be correlated with certain characteristics of the microbial habitats.

The amplicons of the cbbL gene all belonged to form IA RuBisCO. This form is mainly found in Alpha-, Beta- and Gammaproteobacteria, although a few cyanobacterial sequences possess form IA as well (Wielen, 2006). This study also indicated a domination of the Proteobacteria distributed throughout the SCS deep-sea sediments. Giri et al. reported the similar results that the genus *Thiobacillus* and *Thiorhodococcus* were the dominant bacteria isolated from Mono Lake. *Thiobacillus*-related RuBisCO were found to be distributed globally and contribute to primary production in the deep sea (Elsaied and Naganuma, 2001).*Thiocystis* with high proportion was detected in NSCA site, while not detectable in NSCE and NSCI. *Rhodobacter* as one genera of α-proteobacteria was only present at the NSCA site, also not detected in other two sites. The diversity of bacterial populations in marine sediments maybe due to the environmental characteristics difference even in the same habitat. Among the detected groups of the Gammaproteobacteria, the genera *Thioalkalivibrio* were chemotrophic genus, *Halorhodospira* and *Ectothiorhodospira* were phototrophic genus.The 16 phylotypes that we obtained from three SCS deep-sea sediments belong to autotrophic bacteria and most were chemoautotrophic bacteria. This was expected as sampling site is located at 1000 m in the deep sea, a depth at which light does not penetrate. Most of the cbbL sequences detected in deep-sea sediments were found belong to sulfur-oxidizing Gammaproteobacteria and confirm the importance of sulfur cycle bacteria in deep sea ecosystem. *Chromatium*, *Hydrogenophaga* and *Ectothiorhodospira* detected in this study were facultative autotrophic bacteria. In conclusion, we propose that the distribution of the deep-sea RuBisCO genes cbbL may correlate with certain characteristics of the microbial habitats.

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REFERENCES

Coffin M F 2004 Earth, oceans, and life: science of the integrated ocean drilling program (IODP) *Chikyu Monthly* **44** 16

Dai X, Zhou H, Chen YQ 2002 A preliminary study on 16S rDNA diversity of bacteria in the Nansha marine sediment, the South China Sea *Progress in Natural Science* **12** 479

Elsaied H E, Kimura H, Naganuma T 2007 Composition of archaeal, bacterial, and eukaryal RuBisCO genotypes in three Western Pacific arc hydrothermal vent systems *Extremophiles* **11** 191

Elsaied H, Naganuma T 2001 Phylogenetic diversity of ribulose-1,5-bisphosphate carboxylase/oxygenase large-subunit genes from deep-sea microorganisms *Applied and Environmental Microbiology* **67** 1751

Giri B J, Bano N, Hollibaugh J T 2004 Distribution of RuBisCO genotypes along a redox gradient in Mono Lake, California *Applied and Environmental Microbiology* **70** 3443

Hung W L, Wade W G, Chen Y, Kelly D P, Wood A P 2012 Design and evaluation of novel primers for the detection of genes encoding diverse enzymes of methylotrophy and autotrophy *Polish journal of microbiology* **61** 11

Kovaleva O L, Tourova T P, Muyzer G, Kolganova T V, Sorokin D Y 2011 Diversity of RuBisCO and ATP citrate lyase genes in soda lake sediments *FEMS microbiology ecology* **75** 37

Nanba K, King G M, Dunfield K 2004 Analysis of Facultative Lithotroph Distribution and Diversity on Volcanic Deposits by Use of the Large Subunit of Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase *Applied & Environmental Microbiology* **70** 2245

Selesi D, Schmid M, Hartmann A 2005 Diversity of Green-Like and Red-Like Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Large-Subunit Genes (cbbL) in Differently Managed Agricultural Soils *Applied and Environmental Microbiology* **71** 175

Shively J M, Devore W, Stratford L, Porter L, Medlin L, Jr S E S 1986 Molecular evolution of the large subunit of ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) *FEMS microbiology letters* **37** 251

Wielen P W van der 2006 Diversity of ribulose-1,5-bisphosphate carboxylase/oxygenase large-subunit genes in the MgCl2-dominated deep hypersaline anoxic basin discovery *FEMS microbiology letters* **259** 326

Yuan H, Ge T, Chen C, O'Donnell A G, Wu J 2012 Significant role for microbial autotrophy in the sequestration of soil carbon *Applied and Environmental Microbiology* **78** 2328