JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2014. Vol. **8**(3), p. 1981-1984

Low Diversity of Microbial Catalase Genes from Antarctic Surface Seawater off Great Wall Station

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(Received: 19 November 2013; accepted: 08 January 2014)

Catalase plays an important role in the metabolism of Antarctic surface seawater microorganism. To support the hypothesis that the effect of ultraviolet radiation to the surface seawater on the catalase evolution of Antarctic surface seawater microorganisms would lower the microbial catalase diversity, the catalase genes of Antarctic surface seawater samples from three stations were studied by PCR amplification, restriction fragment length polymorphism (RFLP) screening, and phylogenetic analysis. The result showed several unique RFLP patterns in three libraries, indicating that a low diversity of catalase existed in the study areas. Five kinds of microbial catalases including two bacterial catalases and three fungal catalases were confirmed in the Antarctic surface seawater, which manifested the scarcity of microbial catalase diversity.

Key words: Microbial catalase diversity, Antarctic surface seawater, ultraviolet radiation.

Catalase (EC1.11.1.6) is one of the high-active house-keeping proteins in aerobic microorganisms [1], which scavenges H_2O_2 produced in the life process especially the aerobic metabolism to prevent H_2O_2 from oxidizing almost all cell components [2].

The cold environment constitutes a large proportion of the marine ecosystem where the solubility of oxygen is increased. Marine aerobic microorganisms can adapt to consequential increase in reactive oxygen species such as H_2O_2 with their imperative antioxidant catalases [3]. Catalase genes are in the complete genomes of marine cold-adapted bacteria *Desulfotalea psychrophila*, *Colwellia psychrerythraea* and *Pseudoalteromonas haloplanktis* et al, so studies on catalases are of significance to understand the aerobic metabolism of marine microorganisms living at low temperature.

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H_2O_2 concentrations in surface seawater are influenced by ultraviolet, which might reach micromolar concentrations in Antarctic seawater [4]. An oxidative stress situation caused by external H_2O_2 poses a threat to Antarctic marine microorganisms in the surface. Chances are that they have enough catalase activity so as to decompose H_2O_2 and maintain the metabolism [5]. The ultraviolet radiation on the surface seawater as a selective pressure influences the catalase evolution in the microorganisms. It can follow that convergent evolution would lower the microbial catalase diversity.

The microbial catalase diversity in Antarctic surface seawater was detected in this study by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method [6] in order to prove the above hypothesis and improve understanding of the antioxidant capacity of Antarctic marine microorganism. The mechanism for adaption of Antarctic marine microorganism to extreme environment was discussed from the aspect of ecology.

MATERIALS AND METHODS

Sample collection, DNA extraction and PCR

The Antarctic surface seawater samples were collected in March of 2012 (Table 1). Seawater (1 L) was immediately prefiltered through 8-µm pore-size glass fiber filters and then filtered onto 50-mm diameter 0.22-µm polysulfone filter at a pressure <0.03 MPa. The filters were stored for 10 weeks in dark at 4 °C before use. The seawater DNA was extracted as previously described[7]. PCR amplification of microbial catalase gene sequences was performed with the seawater metagenomic DNA as the template[6]. The four employed catalase degenerate primers were as follows: Positive: $C3^+$: GGCACATTTTAATCGAGAACGARTNCCNGARMG; cat+: CCNGARMGNGTNGTNCAYGC;
Negative: $C3^-$: AATCTTTATGAGGCC AAAC TTTTGTNANRTCRAA;
CB^-: AGTAAATGTTCGGCCTTGCARNADYT TRTC.

RFLP and DNA Sequencing

PCR products from six reactions were pooled to minimize PCR bias, gel-purified by Sanprep Kit (Sangon, China) and ligated into pEASY-T1 vectors (Transgen, China) to transform *Escherichia coli* Trans5α competent cells (Transgen, China). Recombinants were selected using X-Gal-IPTG LB indicator plates with 100 µg/mL ampicillin. All the white macroscopic clones were chosen and the positive ones were verified by the catalase gene fragments reamplified from these clones using the degenerate primers, then those positive PCR products were digested by the restriction endonuclease MboI (Thermo, USA). The restriction fragments were resolved by electrophoresis on 1% agarose gels in 10 mM Li^+-H_3BO_3, pH 6.5 [8]. Band patterns digitally photographed with an imaging system (Peiqing Biotech, China) were compared for RFLP analysis to identify redundant clones. One to three clones of every typical RFLP type were chosen and the gene fragments were sequenced (Sangon, China). Catalase sequences were analyzed by BLAST (http://blast.ncbi.nlm.nih.gov) and the phylogenetic groups of the enzymes were determined by MEGA 6 [9].

RESULTS AND DISCUSSION

The diversity of catalase gene from Antarctic surface seawater was shown in Table 1 and Table 2. There were 140 positive clones obtained from the catalase gene libraries from three stations, of which contained 25 RFLP patterns, i.e. unique catalase sequences demonstrated through electrophoresis. Five operational taxonomic units (OTU), however, were confirmed after DNA sequencing of those 25 unique catalases. In all probability, five different kinds of catalases were discovered in the three seawater samples, which were analyzed by BLASTp and categorized separately as bacterial and fungal catalase.

The No. 1 Station sample was first studied among the three, in which the catalase 1-cat1 amplified by the C3+CB- primer pair was detected. This catalase might well derive from γ-Proteobacteria *Marinomonas*. The catalase 2-

Table 1. Biodiversity of the seawater catalase sequences recovered from the three sampling stations off the Antarctic Great Wall Station

Sample sites	Primer pair	No. of screened clones	No. of positive clones	No. of unique catalase sequences	Catalase name (operational taxonomic unit)
No.1 (60°02'W 64°00'S)	C3+CB-	100	32	8	1-cat1
No.2 (60°01'W 61°02'S)	cat+C3-	30	16	3	2-cat1
			10	1	2-cat2
	C3+C3-	68	57	2	2-cat1
			6	2	2-cat2
			4	2	2-cat3
No.3 (62°15'W 60°57'S)	cat+C3-	28	4	4	3-cat1
			2	2	2-cat2
	C3+C3-	39	9	1	2-cat1

cat1 was the most abundant catalase in No. 2 and No.3 sample and was detected by the C3+C3- and cat+C3- primer pairs. The 2-cat1 displayed the highest percentage identity (73-74%) to *Dichomitus squalens* LYAD-421 SS1 catalase, which might be a novel fungal catalase. The 2-cat2 catalase was also detected in No. 2 and No. 3 sample by the C3+C3- and cat+C3- primer pairs, while the 2-cat2 had the highest identity (97-100%) to the *Meyerozyma guilliermondii* ATCC 6260 peroxisomal catalase, a kind of catalase from Ascomycota. The 2-cat3 catalase was only detected in No.2 by the C3+C3- primer pair and might be a novel catalase with highest identity (66-67%) to *Trichoderma virens* Gv29-8 catalase. The 2-cat1 and 2-cat3 might well be both derived from Basidiomycota. No bacterial catalase was discovered in the No. 2 Station sample. The catalase 3-cat1 was only detected in No. 3 sample by the cat+C3- primer pair and belonged to

Table 2. Distribution of operational taxonomic units (OTU) among the sample sites

OTU	n	No. 1	No. 2	No. 3	closest catalase match	Accession
1-cat1	32	32	0	0	*Marinomonas* sp. MED 121	EAQ64286
2-cat1	82	0	73	9	*Dichomitus squalens*	EJF63962
2-cat2	18	0	16	2	*Meyerozyma guilliermondii*	XP_001486179
2-cat3	4	0	4	0	*Trichoderma virens*	EHK18753
3-cat1	4	0	0	4	*Elizabethkingia anophelis*	EHM98381

n is the number of sequences detected for each OTU. Accession numbers represent catalase sequences from cultured microorganism.

Elizabethkingia anophelis Ag1 catalase (99% similarity), a kind of bacterial Flavobacteriaceae catalase. Phylogenetic analysis demonstrated that the five catalases all belonged to the Group III of the monofunctional catalase, which widely spread in all the kingdoms of the life.

Five kinds of microbial catalases including two bacterial catalases and three fungal catalases were detected in the Antarctic surface seawater, which manifested the scarcity of microbial catalase diversity. Because the Antarctic ultraviolet radiation in summer was strong enough to enforce the selective pressure on the microorganism dwelling in the surface seawater, several catalases with high activity predominated in the microbial community of surface seawater while the less active ones were replaced. As a result, the variety of catalase decreased, which caused low diversity level of catalase. The result of this study was in accordance with our presupposition; however, it was worth noting that such speculation demanded further experimental evidence. The catalases obtained in this study were just gene fragments of which the activity of decomposing H_2O_2 could not be measured. So we plan to clone and heterogeneously express the complete catalase genes in order to measure the catalase activity in future.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (NSFC) grant 41006119.

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