Heavy Metal Tolerance of An Antarctic Bacterial Strain O5 and Its Antioxidant Enzyme Activity Changes Induced by Cu^{2+}

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***Abstract*—Under the heavy metal polluted circumstances, microorganisms certainly have some changes in terms of species, quantity, community structure and diversity to adapt the environments. Now, many heavy metal tolerant microbe groups have been studied. In the study, a heavy metal tolerant and psychrophilic bacterium strain from Antarctica was screened. Based on 16S rDNA sequence analysis, this strain belongs to *Planococcus*, named as *Planococcus* sp. O5. The capacity of antimetal of *Planococcus* sp. O5 is $Pb^{2+} > Cu^{2+} > Hg^{2+} > Cd^{2+} > Zn^{2+}$, and the MICs is 320 mg/L, 130 mg/L, 80 mg/L, 80 mg/L and 40 mg/L, respectively. Lipid peroxidation (indicated by malonydialdehyde content) happened in strain O5 induced with Cu^{2+}. At the same time, the antioxidation enzyme activity (such as SOD, POD and CAT) had stimulus-controlled improvement, which is a certain protection against heavy metals. Therefore, as an important feature adapting the stress environments, the activity of antimetal, can reflect the adaptive strategy of microorganism to some extent. This paper studied the activity of antimetal and antioxidation of a bacterial strain, which can help us better understand the bacteria how to adapt the extreme environments.**

Keywords-Antarctic bacterium; 16S rDNA analysis; heavy metal tolerance; antioxidation enzyme system

I. INTRODUCTION

Up till the end of last century, lots of heavy metals were released to our surroundings every year. Heavy metals can be absorpted by organism, and then reach a very high enrichment at the end of food chain, which can cause various malformation, toxicosis, and even canceration and so on. The pollution of heavy metals has been a worldwide problem, and Minamata disease and Itai disease happened in Japan were caused by the severe exceed of Hg and Cd in the environment.

Antarctic continent retains original environment, have the characters of extreme environments, such as low temperature, low illumination, oligotrophic and intenseradiation, etc. Now, Antarctic is no longer pure land, and frenquent human activities has influenced Antarctic environment seriously. The pollution degree of heavy metals in Antarctic exceeds the imagination of human being far away, and heavy metals enriched in the organism is even more than that from other oceans[1,2,3]. Recently, the international academic and governments have maken various methods and protect regulations and done lots of research and evaluation about the influence of the Antarctic environment[4].

The pollution of heavy metals becomes more and more serious, the migration and accumulation of heavy metals to Antarctic has become one of the severe problems of Antarctic. Recent scientific research indicates Atmospheric circulation and atmospheric sedimentation are the major way for heavy metals entering the Antarctic ecosystem[5,6], which can cause the accumulation of heavy metals in Antarctic[7,8]. Heavy metals can produce adverse impact on organism through the physiological poison at the level of individual, population, species, communities and so on. In recent decades, the content of heavy metals in Antarctic ecosystem has increased, for instance, Pb、Ba、Cd、Hg and so on, which can affect the tender ecosystem greatly[9-13]. So the pollution of heavy metals becomes a severe problem to be solved.

In this study, a bacterial strain named *Planococcus* sp. O5 was screened from sea ice of Antarctic for its high resistance against metals and some antioxidation enzyme activity were also experimented.

II. MATERIALS AND METHODS

A. Microorganisms

A bacterial strain *Planococcus* sp. O5 screened from sea ice in Antarctica was used throughout this study.

B. Strain culture

2216E medium (0.5% peptone, 0.1% yeast extract, 0.0015% $FePO_4 \cdot 4H_2O$) was used for culture of *Planococcus* sp. O5. Heavy metals (Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, the concentration gradient is 0, 5, 10, 20, 40, 80, 120, 160 mg/L, 120, 160, 240, 320, 400, 480 mg/L is extra for Pb^{2+}) was used to detect the heavy metal tolerant capacity.

C. Morphological and molecular identification of bacterium

The morphological characteristics of the bacteria were observed through microscope after cultured at 10℃ on 2216E medium based on Gram staining. The bacteria was splitted for 20 minutes in water bath at 100℃, then conserved at 4℃ and the molecular identification of the bacteria was performed through 16S rDNA sequence analysis. The 16S rDNA sequence was determined by using primers 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-AAGGAGGTGATCCAGCCGCA-3'. PCR was performed

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under the following conditions: 95℃ for 5 min, followed by 94℃ for 45 s, 55℃ for 45 s, and 72℃ for 90 s for 30 cycles and then 72℃ for 10 min. The PCR products were further analyzed by sequencing at Shanghai Sunny biotechnology Co.,Ltd, and then nucleotide alignments, at last, constructed a philosophy tree of *Planococcus* sp. O5 based on Neighbor-joining method.

D. Assay of activity of antimetal

Measure OD_{595} for each metal at each concentration for 3 times after *Planococcus* sp. O5 were cultured at 10℃ for 7 days at 2216E liquid medium, 2216E liquid medium without heavy metal as the control.

E. Membrane lipid peroxidation assay

Level of membrane lipid peroxidation is indicated with malondialdehyde (MDA) content. MDA content was determined by using TBA and TCA[14].

F. Assay of antioxidation enzyme activity

After 10 days culture with and without Cu^{2+} (0.5 mmol/L) respectively, strain O5 was collected by centrifugation (6000 r/min, 15 min at 4℃). The precipitation was ground in PBS buffer (pH 7.0) to break cells. The grinding fluid was centrifuged with 12000 r/min for 20 minutes at 4℃, and the supernatent was kept at -40℃ to determine antioxidation enzyme activity.

The determination of SOD activity was according to Zhang *et al*[15].A unit of the enzyme activity is defined as the enzyme quantity when the inhibition rate of the oxidation of pyrogallol is 50%. The assay of POD activity refered to the method of Zou *et al*[16]. A unit of the enzyme activity is defined as OD_{470} change of 0.01. CAT activity is determined by reaction with H_2O_2, then measure its OD_{240}[17]. A unit of the enzyme activity is defined as OD_{240} change of 0.1.

III. RESULTS

A. Identification of bacterium

Morphological observation showed that the cell is round, bacterial colony is yellowish orange, and the strain is gram-positive. Analysis of 16S rDNA sequence of the bacteria and the blast search of the GenBank database demonstrated that the sequences exhibited 99% sequences similarity with the 16S rDNA gene of some strains. So, we constructed a phylogenetic tree of the strain based on Neighbor-joining method (Fig.1). This result indicates that the strain belongs to *Planococcus***.**

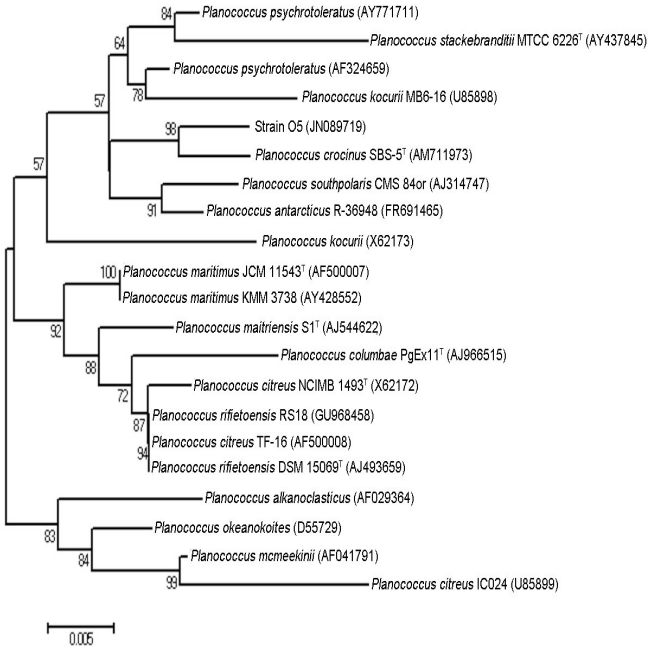

Figure 1. The phylogenetic tree of bacteria based on Neighbor-joining method

TABLE I. GROWTH SIATUS OF STRAIN O5 IN DIFFERENT CONCENTRATIONS AND DIFFERENT METALS(OD_{595})

Heavy metals	Induced concentration (mg/L)							
	0	***5***	***10***	***20***	***40***	***80***	***120***	***160***
Cu^{2+}	1.772±0.031	1.763±0.048	1.702±0.009	1.700±0.019	1.786±0.028	1.380±0.015	0.101±0.022	0.093±0.013
Zn^{2+}	1.772±0.031	1.700±0.064	1.557±0.035	1.200±0.074	0.263±0.075	0.158±0.046	0.185±0.033	0.185±0.008
Hg^{2+}	2.046±0.071	2.081±0.057	1.985±0.006	2.056±0.070	1.912±0.004	0.093±0.044	0.064±0.005	0.078±0.002
Cd^{2+}	1.800±0.039	1.803±0.037	1.611±0.183	1.643±0.050	1.019±0.481	0.229±0.070	0.128±0.117	0.125±0.034
Pb^{2+}	2.001±0.055	1.864±0.014	1.995±0.147	2.040±0.092	2.021±0.035	1.916±0.041	1.807±0.019	1.818±0.021

B. Assay of activity of antimetal

Table Ⅰ showed the growth status of *Planococcus* sp. O5 stressed with different heavy metals of different concentration. It can be seen this strain still grew uneffectly induced with Pb^{2+} of 160 mg/L, so higher concentration gradient (120, 160, 240, 320, 400 and 480 mg/L) was done and the results were showed as Table Ⅱ. As a conclusion, we found the minimum inhibitory concentrations (MICs) of each metal for *Planococcus* sp. O5, is Pb^{2+}>Cu^{2+}>Hg^{2+}>Cd^{2+}>Zn^{2+}, and the MICs was 320 mg/L, 130 mg/L, 80 mg/L, 80 mg/L, 40 mg/L. So, the bacteria has a strong capacity of antimetal, especially against Pb^{2+} and Cu^{2+}, the result was verified by fresh weight experiment.

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TABLE II. GROWTH STATUS OF STRAIN O5 IN DIFFERENT CONCENTRATIONS OF DIFFERENT METALS (OD_{595})

Heavy metals	Induced concentration(mg/L)						
	0	*120*	*160*	*240*	*320*	*400*	*480*
Pb^{2+}	1.599± 0.012	1.702± 0.077	1.809± 0.157	1.438± 0.475	0.724± 0.036	0.764± 0.107	0.751± 0.003

C. *Assay of activity of antioxidation*

From Fig.2, we found the activity of antioxidant enzyme was maximizing on about the second day, then maintained a stable level, the degradation product MDA of polyunsaturated fatty acid superoxide was maximizing on the fourth day, reduced till the fifth day, then maintained a stable level. So, we found the protect response was delayed.

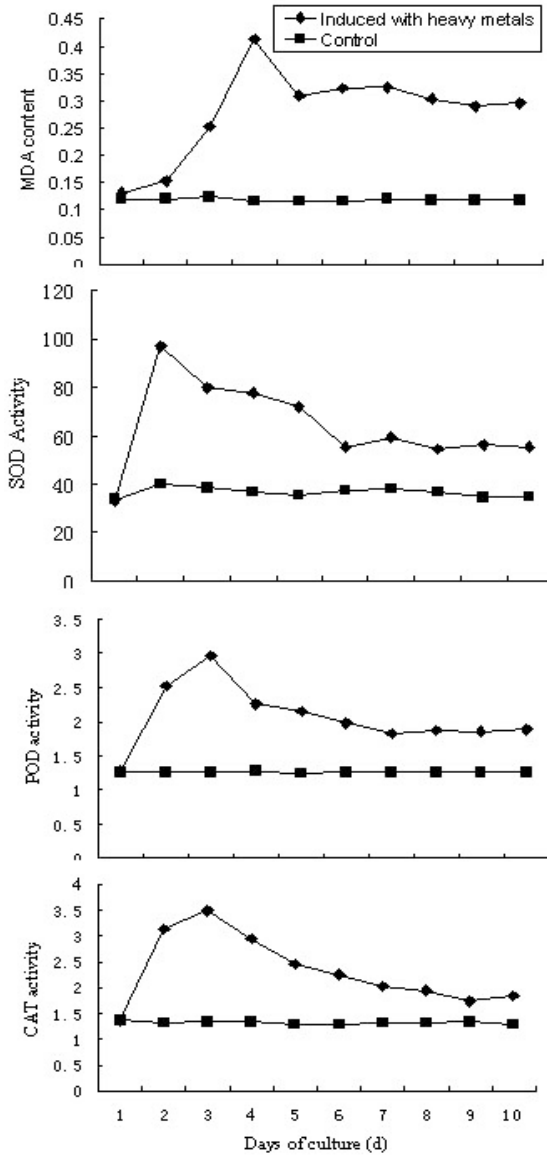

Figure 2. The changes of antioxidant enzyme activity and MDA content in strain O5 induced with 0.5 mM Cu^{2+}

IV. DISCUSSION

Heavy metals have strong biological toxicity, and they can affect the natural ecosystem even in very low concentrations. In addition, heavy metals have the character of being latent and protracted and irreversible. Heavy metals can cause the change of physiological and biochemical indexes, destroy the structure of life macromolecules (e.g. protein and DNA), and induce malformation and apoptosis of cells. Through adsorbing in the surface of organs or into the organism, these metal ions can influence the function of enzyme activity, metabolism and inheritance.

Management of waste water and soil with heavy metals is one of the important topics in the field of environmental protection. The traditional processing method involves chemical precipitation process, electrolytic process, ion exchange process, physical adsorption process[18]. However, these processes all have shortcomings, e.g. high cost, easy to cause the second pollution and so on. Especially heavy metals concentration is certain lower, operating cost and raw material cost is relatively high. In recent years, removal of heavy metals from waste water through biological adsorption method has been reported[19,20,21]. In the environments of low concentration heavy metal, absorbent of microbial sources have the characteristics of high efficiency, large absorption capacity, fast, simple-equipmented and so on. Therefore, biological adsorption method shows broad application prospects in the field of heavy metal removal. Commonly, various heavy metal pollutants coexist with a large number of organic pollutants. So further researches are necessary if the strain is applied to deal with the polluted environments with heavy metals.

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