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# Effect of Lead on Superoxide Dismutase, Catalase and ATPase Activity in Lead Resistant Bacteria, *Bacillus cereus*

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**Studies were undertaken to investigate the activity of Superoxide Dismutase (SOD), Catalase (CAT), ATPase and the SOD isoenzyme patterns in a lead resistant bacteria *Bacillus cereus* isolated from the industrial effluent contaminated with lead. The activities of SOD and CAT in *Bacillus cereus* increased with increasing concentrations of lead under aerobic conditions, reaching the highest level with 500 mg/l and 10,000 mg/l of lead, respectively. Lead also showed an evident influence on ATPase activity in the bacteria. Two SOD isoenzymes were detected in the bacteria by native PAGE analysis. It is thus presumed that lead causes oxidative stress which increases SOD and CAT activities, and also generates SOD isoenzyme in lead tolerating bacteria to antagonize the oxidative stress.**

**Key words:** SOD, CAT, ATPase, Lead, *Bacillus cereus.*

Heavy industrialization during the past few years has given rise to serious problems of environmental contamination owing to the lack of safe procedures for effluent treatment and disposal of toxic substances. The wide scale application of a variety of heavy metals in industries has resulted in contamination of the natural ecosystem. Presence of heavy metals in the environment has attracted a great deal of attention due to their toxic nature, translocation and magnification through the food chain.

Lead is a heavy metal found widely in nature with no specific biological function. Recent studies indicate that lead may cause oxidative stress (OS) via generation of reactive oxygen species (ROS)[5,15,19]. These ROS are highly active and found to cause enzyme inactivation, protein denaturation, lipid peroxidation and DNA mutation resulting in ecotoxicity through oxidative damage to cellular components[8,21]. Inci Ergurhan – Ilhan *et al.*[10] have reported that chronic indirect lead exposure results in lipid peroxidation in erythrocytes. Therefore elimination of superoxide anion is absolutely necessary for the survival of cells.

In microorganisms, ROS are produced not only during aerobic growth but also under different abiotic stresses. Antioxidant enzymes such as SOD and CAT protect biomolecules from ROS mediated damage in vivo by maintaining lower concentration of oxygen radicals in the cell. In resistant forms, stress conditions may enhance protective process through activation of the detoxifying enzymes. Xiao- Huo Yao *et al.*[22] reported that acetamiprid causes oxidative stress in bacteria, which not only increases SOD and CAT activities but also generates new SOD isoenzymes to antagonize the oxidative stress.

ATPase plays an important role in many intracellular physiological functions and is likely to undergo series of changes under stress conditions. Yadwad *et al.*[24] pointed out that ATPase can be considered as a sensitive indicator of toxicity. Daivis *et al.*[4] reported that pesticide

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inhibits the activity of $NA^+ K^+$ -ATPase in rainbow trout. Sancho *et al*.[17] reported that fenitrothion influenced ATPase activity in gill tissue of eel. Lu *et al*.[13] showed that ATPase activity in *Burkholderia cepacia* WZI changes significantly after exposure to herbicide, Quinclorac.

Resistance to lead has been reported in some bacteria such as *Pseudomonas margina*lis, *Bacillus megateriu and Chryseomonas luteola*[1]. The effect of heavy metal stress on SOD activity has been studied in Cyanobacterium, *Spirulina platensis -S5*[14]. There has been sufficient work carried out on the response of SOD and CAT activity in bacteria exposed to Xenobiotics like Acetamiprid[22], Quinclorac[13], Bensulfuron-methyl[23], Paraquat[25] and Vinclozolin[6].

But the available literatures do not envisage any information on the response of lead resistant bacteria to heavy metal stress. Hence in the present study, an attempt has been made to investigate these responses on antioxidant defense enzymes, such as SOD and CAT and also ATPase which is known to undergo changes under stress conditions, in a lead resistant bacteria, isolated from the industrial effluent during the course of this investigation. This report is the first demonstration of oxidative (stress) enzymes in lead resistant bacteria.

## MATERIAL AND METHODS

### Isolation of Lead resistant bacteria from water sample

The Vrishabhavathi River, flowing through Peenya Industrial area, Bangalore, (Karnataka, India) has dried up and now carrying industrial effluent and urban sewage, was selected for sample study. 100 ml of water samples were collected from four different locations of the Vrishabhavathi River. The water sample was passed through a sterile membrane filter (0.45 µm pore size) and then the membrane was aseptically placed on the surface of tryptone glucose extract agar medium, supplemented with 100 mg/l of lead, for the isolation of bacteria. Plates were incubated at 37°C for 24 hours. The growing colonies were identified, isolated and purified. The MICs of lead was determined for the isolated colonies and the isolate showing highest MIC, was identified and confirmed as *Bacillus cereus* (GenBank Accession Number: EF488087) at Bangalore Genei, Bangalore based on 16S rDNA data.

### Culture conditions

*Bacillus cereus* was grown aerobically in tryptone glucose extract agar broth at 30°C, and at 150 rpm for 24 hours. After 24 hrs, the cells were harvested from the liquid culture by centrifugation, washed twice with deionized water and the pellet was suspended in 10 ml deionized water, supplemented with different concentrations of lead (100,200,300,400,500,1000 and 10,000 mg/l). The suspended pellets were then incubated for 24 hrs before harvesting for extraction of enzymes.

### Cell harvest and lysis procedure

The pellets obtained above, were re-suspended in 50 mM phosphate buffer (pH) solution (5ml) and then subjected to sonication in an ice-cold water bath for 10 minutes. Cell debris was removed by centrifugation at 10,000 g at 4°C. The supernatants were collected for activity assays, native PAGE and activity staining of SOD. Total protein concentration in cell lysate was determined according to the method of Lowry *et al.* [12], using BSA as the standard.

### Measurement of enzyme activity

The SOD activity was determined by monitoring the inhibition of photo-chemical reduction of nitroblue tetrazolium chloride (NBT), using a reaction mixture consisting of 78 µM NADH, 50 µM NBT, 10 µM PMS and 16 mM Tris- HCl buffer (pH 8.0) and the absorbance was recorded at 560 nm.

The catalase activity was determined according to the method of Worthington Biochemical Corporation where 1 unit of CAT was defined as the amount of lysate that decomposed 1 µM of $H_2O_2$ per mg of protein at pH 7.0 and 25°C in 1 min. The reaction mixture consisted of 0.059 M Hydrogen peroxide in 0.05 M Potassium phosphate (pH 7.0) and 0.1ml 0f diluted enzyme.

The ATPase activity was measured by determining the release of inorganic phosphate from ATP using the procedure of Taussky & Shorr[20]. The assay mixture consisted of 100 mM Tris -HCl buffer, 4 mM ATP, 4 mM $CaCl_2$ and cell free extract. The enzyme activity is expressed as µ mole inorganic phosphate (pi) librated per mg protein in 15 min under the standard assay conditions.

**Native Gel Electrophoresis of SOD and Activity Staining**

The cell lysate was loaded onto an 8% native polyacrylamide gel bathed in 1×Tris-glycine buffer (pH 8.3) and the proteins separated at constant current (20 mA) at 4°C. SOD activity staining of the gel was performed by the method of Rao *et al.*[16]. The gels were stained by incubating in a solution containing 2.5 mmol/L nitro-blue tetrazolium (NBT) in dark for 20 min, followed by incubation in 50 mmol/L potassium phosphate buffer (pH 7.8) containing 28 mmol/L riboflavin and 28 mmol/L tetramethylethylene- diamine in dark for 15 min. The gels were then placed in 50 mmol/L potassium phosphate buffer (pH 7.8) containing 100 µmol/L EDTA and exposed to light (4000 Lx) for 20 minutes at room temperature. The gels were kept in 7.5% glacial acetic acid at 4°C until photographed[3].

## RESULTS

**MIC of lead on *Bacillus cereus***

The MIC of lead was determined using increasing concentrations of Lead (100,200, 300, 400, 500, 1000 mg/l). The result in Fig. 1 revealed that MIC of Lead for *Bacillus cereus* (Isolate A) was at 200 mg/l, where as the other bacteria showed less MICs.

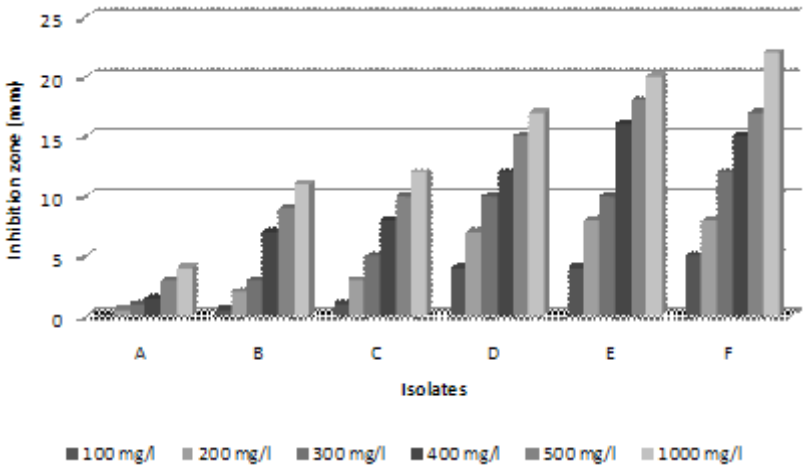


**Fig.1.** MIC of Lead in isolates A,B,C,D,E & F

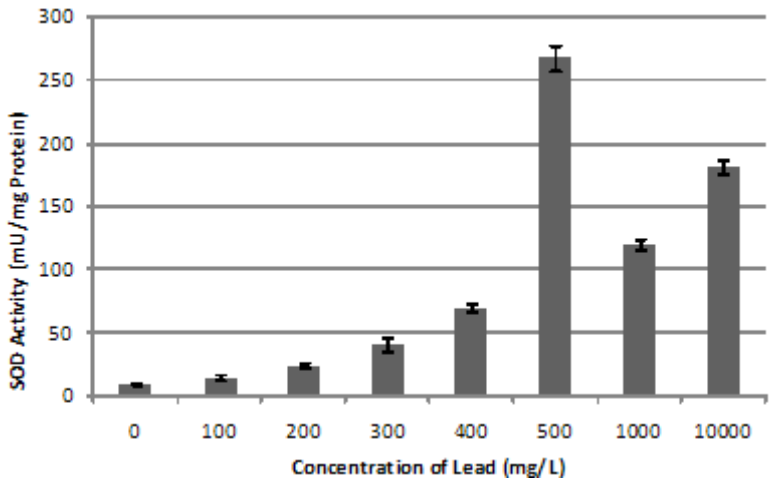


**Fig. 2.** Effect of different concentrations of Lead on SOD activity in *Bacillus cereus*

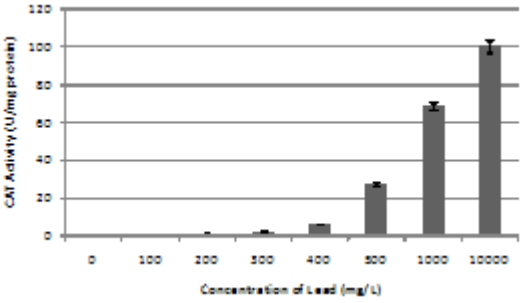


**Fig. 3.** Effect of different concentrations of Lead on CAT activity in *Bacillus cereus*

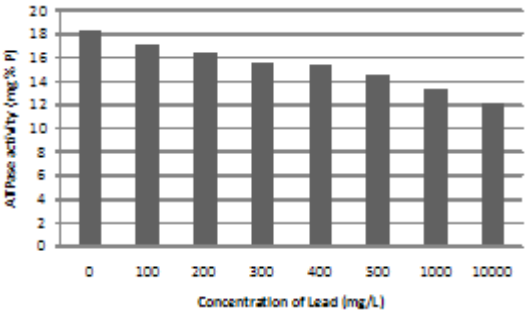


**Fig.4.** Effect of different concentrations of Lead on ATPase activity in *Bacillus cereus*

**Effect of lead on SOD, CAT and ATPase activities**

The activities of SOD, CAT and ATPase in *Bacillus cereus* treated with different concentrations of lead is presented in Fig.2, 3 and 4 respectively. As shown in Fig.2, the SOD activity was stimulated after lead application and reached the highest level (267.5 mU/mg protein) with 500 mg/L of Lead. Percentage increase was maximum with lead ion, compared to the control value of 9.7 mu/mg protein. The results shown in Fig.3 indicate that CAT activity of *Bacillus cereus* increased in a concentration dependent manner with 10,000 mg/L Lead showing the highest activity of 100 Units / mg protein.The effect of lead on ATPase activity in *Bacillus cereus* is summarized in Fig.4. The ATPase activity decreased significantly when the bacteria was grown in medium with lead compared to the control. The result implied that lead inhibited ATPase activity in the bacteria.

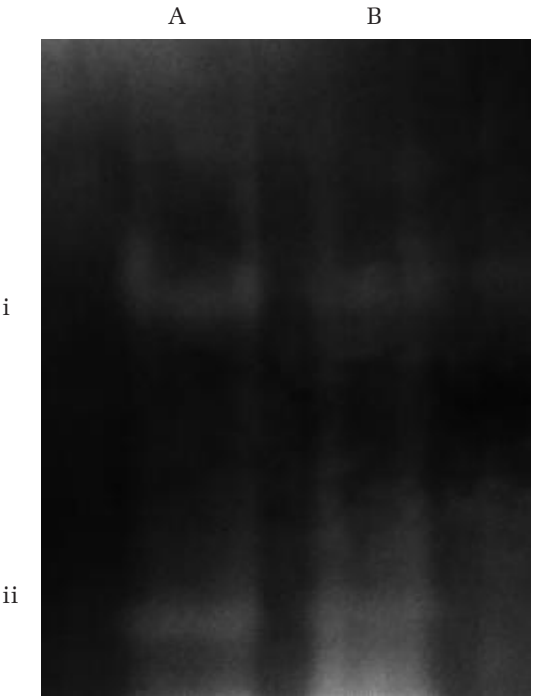


**Fig. 5.** Gel activity staining of *Bacillus cereus* for SOD isoenzyme profile. Lane A -500 mg/L Lead and Lane B- 1000 mg/L Lead

**Effect of lead on SOD isoenzyme patterns**

Samples of bacterial cell lysate treated with 500 mg/L (SOD activity of 267.5 mU/mg protein) and 1000 mg/L (SOD activity of 120 mU/mg protein) lead were used for native PAGE and gel activity staining of the SOD along with the control. The isoenzyme pattern in the bacteria were examined to determine the production of SOD enzyme under oxidative stress induced by lead. Native PAGE analysis (Fig.5) indicated that two SOD isoenzyme bands (i and ii) appeared in samples treated with 500 (lane A) and 1000 mg/L (lane B) of lead, with no band appearing in the control sample.

## DISCUSSION

Recent studies indicate that lead causes oxidative stress (OS) via generation of reactive oxygen species (ROS)[5, 15, 19]. Formation of reactive oxygen intermediate outstrips the scavenging of anti oxidant defense mechanisms, leading to accumulation of free radicals and increase in the oxidative damage to macromolecule (eg. enzymes,

proteins, DNA and membrane lipids)[7]. It is also known that the cell membrane is the target for oxidative damage due to heavy metal exposure. But these studies mainly focus on damages to animals. All aerobic organisms have the natural protective systems of defensive enzymes and endogenous antioxidants which are capable of scavenging active oxygen species below damaging levels. CAT and SOD are known to be the most efficient defensive enzymes against reactive oxygen species (ROS)[18].

The present study investigates the activity of enzymes under oxidative stress induced by lead in *Bacillus cereus,* isolated from Vrishabhavati River that has been contaminated with industrial effluents. It is observed that the activity of CAT increased in a concentration dependant manner (Fig.3) after application of lead, whereas SOD activity attained the peak with 500 mg/L of lead. This increase in the enzyme activity suggests that lead causes oxidative stress leading to more ROS production and in order to antagonize the ROS, defensive enzyme systems such as CAT and SOD activities are enhanced. On the other hand, ATPase activity decreased (Fig.4) after lead application suggesting that lead may affect the metabolism of ATP to a certain degree. Thus, it is presumed that *Bacillus cereus* has developed resistance against lead perhaps through enhancing the activities of both CAT and SOD enzymes.

The high activity of SOD (Fig.2) in *Bacillus cereus* was further found to be associated with the existence of two isoforms of the enzyme, as detected through the native PAGE (Fig.5). Bacterial SOD enzymes have been extensively studied in *E.coli* and it has been shown that this gram negative bacteria possesses two cytoplasmic SOD enzymes, one with Iron (Fe-SOD) and the other with Manganese(Mn-SOD) as the cofactor, respectively and a periplasmic SOD with Copper and Zinc (Cu/Zn SOD) as the cofactor[11]. In contrast, *Bacillus cereus,* a gram positive bacteria possess two isoforms of SOD. Further studies on the characterization and functional assignment of these two isoforms of SOD in *Bacillus cereus* are necessary to draw a definite conclusion.

From the present study it can be summarized that presence of lead in the environment causes oxidative stress. Micro-organisms develop resistance to lead through enhancing the activities of oxidative enzymes such as CAT and SOD. As evidenced and made as a first report in this study, *Bacillus cereus*, a supposedly lead resistant bacteria isolated from the industrial effluent, showed higher activities of both the CAT and SOD enzymes which probably confer its resistance to lead. However, the precise action of lead on microorganisms and the role of antioxidant enzymes in the defense mechanisms of bacteria against lead need to be further investigated.

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