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# Cobalt has Enhancing Effect on Extracellular Lipases Isolated from *Pseudomonas aeroginosa* JCM5962(T)

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**Abstract :** Present study demonstrates effect of metal ions and inhibitors on the activity of lipase, isolated from *P.aeruginosa*. Seven different concentrations (0.1mM - 20mM) of twelve metal salts and two inhibitors, EDTA and SDS were studied for their effect on lipase. The results demonstrated that ten metal ions, namely$Fe^{+2}$, $Zn^{+2}$,$Ba^{+2}$, $Na^{+}$, $NH_4^{+}$, $Mg^{+2}$,$K^{+}$, $Li^{+}$, $Ca^{+2}$and$Cu^{+2}$reduced the activity of lipase enzyme at a concentration of20mM;where some of these moderately increased the activity and then suppressed. While Mercury completely inhibited the enzyme at a concentration as low as 0.5mM; Cobalt showed significantly activating effect on it. Both had their effect in a dose dependent manner and all concentrations of $CoCl_2$ enhanced lipase activity, with a 58% increase of initial activity at 20mM concentration. $Na^{+}$, $NH_4^{+}$,$Ba^{+2}$,$Li^{+}$,$Mg^{+2}$ and$K^{+}$ were not found to be significantly affecting the enzyme. The enzyme was also tested for two inhibitors, EDTA and SDS; both reduced the enzyme action and SDS showed complete inhibition at highest concentration, suggesting the dependence of enzyme on metal ions. Lipases, being one of the most extensively used industrial enzyme, a stable variant from a microbial source may be of immense potential.
**Key words:** Lipase, Mercury, Cobalt, *Pseudomonas*, Metal ions, chelating agents.

## Introduction

Lipases (triaclglycerol hydrolases, EC 3.1.1.3) are hydrolases acting on the carboxylic ester bonds exist in acylglycerols to liberate fatty acids and glycerol. These are among the most important industrial enzymes in terms of their versatility[1;2]. They are found in wide diversity of sources such as blood, gastric juices, adipose tissues, intestinal juices and pancreatic secretion. Alkalophilic and thermostable lipases have great potential to be used in food flavoring, detergent[3], leather processing, pharmaceutical, cosmetics[4]. Lipases are of significant importance in leather industry. Lipases remain enzymatically active in organic solvents[5]that enhance their potential and flexibility as biocatalysts against a wide range of unnatural hosts[6]. Microbial lipases are usually extracellular enzymes, which are produced by various bacteria, actinomycetes, yeast and fungi[7]. Some of the lipolytic bacterial species are *Pseudomonas fragi, Bacillus subtilis, Bacillus megaterium, Pseudomonas pseudoalcaligenes, Staphylococcus aureus, Burkholderia cepacia and Pseudomonas aeruginosa.* Some common lipase producing fungal species are *Helvina lanuginosa, Rhizopus delemar, Eurotrium herbanorium, Aspergillus niger, Mucor circinelloides and Penicillium citrinum* etc.



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Lipases are capable of catalyzing reactions at low and moderate temperatures and hold a great potential in certain areas of industries such as synthesis of heat liable compounds, detergents and biodiesel production. They are referred as highly specialized proteins and are reaction catalyst of biological systems. Enzymes are one of the keys to understand how cellssurvive and proliferate.Heavy metal ions are strongly bound by sulfhydryl groups of proteins. Sulfhydryl binding changes the structure and enzymatic activities of proteins and cause toxic effects evident at the whole organism level. The use of heavy metals such as mercury, copper, lead, cadmium in agriculture and industrial hasincreased tremendously.The heavy metals have specific binding affinity tosulfhydryl group of the enzymes. They alter the activity of enzymesthat range fromactivation to total inhibition.The metal induced alteration in enzyme activity may be taken as more or less accurateindicator of metal toxicity[8].

The aim of the present study was to examine the effect of metal ions at different concentrations,on the activity of lipase enzyme.The acute effect of two inhibitors at different concentrations was also examined.

## Materials and Methods

### Microorganism and enzyme production

*Pseudomonas aeruginosa* isolated from the soil of sugarcane crop field was used in the study. The samples were grown on the tributyrin agar medium and the colonies which show a wide clear zone have been isolated and stored for further use.For the production of lipase,bacterial colonies were cultured in nutrient mediumin shake flask cultures for 24 hr at 37$^{o}$C. Enzyme is extracted by centrifugation at 10,000 rpm at 4°C for 10 min and activity of lipase was checked as given below.

### Lipase assay

Lipase activity was assayed using p-nitrophenylpalmitate as a substrate according to the protocol as described byGhoriet al[9]. As described above enzyme is extracted by centrifugation at 10,000 rpm at 4°C for 10 min. Clear supernatant obtained was used as crude enzyme and stored in sterilized vials for further use. Substrate solution containing phosphate buffer with gum Arabic (Hi Media) and sodium deoxycholate (Sigma Aldrich, USA) along with p-nitrophenylpalmitate (Sigma Aldrich, USA) in isopropanol was pre-incubated with crude enzyme at 37°C.The reaction was stopped by adding 0.5 mL of 3M HCl and centrifuged at 10000 rpm for 10 minutes at room temperature. The release of p-nitrophenol (*p*NP) was measured spectrophotometrically at 405 nm. One unit of lipase activity was defined as the amount of enzyme releasing 1 µ mol *p*NP under standard assay condition.

### Effect of metal ions and inhibitors

A total of twelve metal and two inhibitors were randomly selected for the study. The metal salts used in the study were$FeSO_4$, $CuSO_4$,$ZnSO_4$, $CaCl_2$, $CoCl_2$, $HgCl_2$, $BaCl_2$, NaCl, $Nh_4Cl$, $MgCl_2$, KCL,LiCl, whereas the inhibitors were EDTA (Ethylenediaminetetracetic acid) and SDS (sodium dodecyl sulphate). The effect of metals and inhibitors was studied by incubation ofenzyme in the presence of different concentrations of metals or the inhibitor according tomodified method of Siddiquiet al[10]. Seven different concentrations (0.1 mM, 0.2mM, 0.5 mM, 1 mM, 5 mM, 10 mM, 20mM) were prepared by dissolving appropriate amount in double distilled water. Equal amount of enzyme extract and test solution were mixed and incubated for 30 minutes at 37$^{o}$C before testing the lipase activity. The enzyme activity in each test solution was determined by normal lipase assay procedure as discussed above.

## Results

The effect of metal ions viz.$Fe^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Ca^{2+}$, $Co^{2+}$, $Hg^{2+}$, $Ba^{2+}$, $Na^{+}$, $NH_4^{+}$, $Mg^{2+}$, $K^{+}$, $Li^{+}$and two potential inhibitors EDTA and SDS was studied on microbial lipase enzyme. A rich source of extracellular lipase was isolated from soil and identified to be *Pseudomonas aeruginosa*(data not presented here).The bacterium was incubated for 24 hr at 37ºC for enzymeproductionand extracellular lipases were extracted and used in the study. All of the twelve metal salts and inhibitors had been tested in seven different concentrations, starting from 0.1mM to 20mM.The effect on lipase enzyme was calculated as % decrease or increase and presented in Table 1. All salts tested during the study inhibited the enzyme activity at various levels, except one

i.e. Cobalt. Majority of the metals did not have very noticeable effects at very low concentrations (0.1mM to 5mM) and the decrease/increase in enzyme activity became significant only from 10mM onwards.The results, presented in figure 1 demonstrate that four metal ions:iron, zinc, calcium and copper significantly reduced the activity of lipase enzyme at a concentration 20 mM(upto80% loss of activity). Inhibitory effect was also obtained with Ammonium, barium, sodium, potassium, lithium and magnesium; but not to that extent (4-30% loss in activity). Mercury in our study was found to be highly toxic on lipase as it completely inhibited the activity at the lowest tested concentration of 0.05mM( >90% loss of enzyme activity).

It is now well established that in addition to common metals like sodium and iron, many trace metals like copper, zinc, molybdenum, cobalt etc. are also essential in nutrition. Many of these metals including trace metals have been demonstrated to have activating effects. A similar significant finding of the present study was the enhancing effect of Cobalt on lipase enzyme. Figure 2 shows the percent enhancing effect of$Co^{2+}$as well asinhibiting effect of $Hg^{2+}$and SDS. At highest test concentration of 20mM, cobalt significantly enhanced the enzyme activity and a 55.6% increase in lipase activity was obtained.Of the two tested inhibitors, EDTA reduced lipase activity to 38% at 20mM concentration; butSDS(at20mM) completely inhibited the activity to less than one.

**Table 1: Percent Inhibition / Increase in Enzyme Activity at Different Concentrations of Some Metals**

| **Metals →** | $Zn^{2+}$ | $Fe^{2+}$ | $Ca^{2+}$ | $Cu^{2+}$ | $Mg^{2+}$ | $Hg^{2+}$ | $Co^{2+}$ |
|---|---|---|---|---|---|---|---|
| **Concentrations** | **% Inhibition** | | | | | | **% Increase** |
| 0.01 mM | -2.12% | -3.4% | -5.38% | -1.08% | -1.5% | 1.9% | 6.5% |
| 0.02 mM | -4.46% | -4.7% | -6.84% | -0.58% | -2.0% | 6.5% | 11.8% |
| 0.05 mM | -3.5% | -5.6% | -4.12% | 4.7% | -3.14% | 20.7% | 25.5% |
| 1 mM | 1.08% | 2.36% | -1.86% | 13.45% | -0.51% | 32.5% | 33.7% |
| 5 mM | 5.02% | 7.66% | 2.39% | 55.03% | 2.3% | 51.3% | 55% |
| 10 mM | 22.87% | 16.84% | 17.18% | 68.56% | 6% | 76.7% | 55.6% |
| 20 mM | 57.91% | 44.69% | 43.8% | 82.3% | 30% | 99.15% | 55.6% |

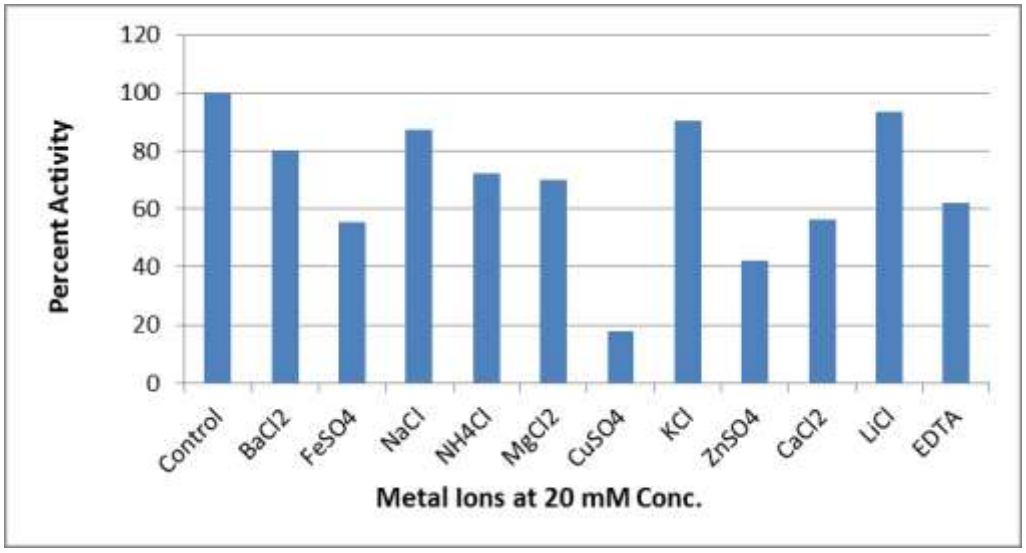


**Fig. 1: Effect of metal ionsand inhibitors on Lipase activity**

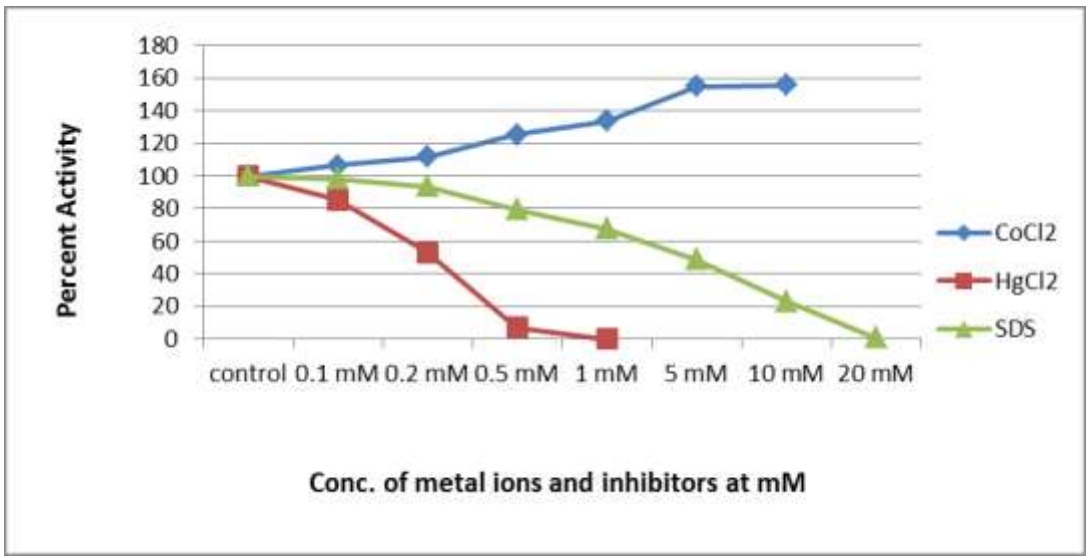


**Fig. 2: Effect of Cobalt, Mercury and SDSon lipase activity**

## Discussion

Metals are known to have important biological role in enzyme function. They can affect the enzyme activity by a number of modes, viz. serving as electron donor or acceptor, forming enzyme bridge complexes and structural regulators. Inhibition studies give important insight into the nature of an enzyme, its cofactor requirements and nature of the active site[11](Sigma & Mooser, 1975). The present study demonstrated the role of various activators (metal ions and inhibitors) in enhancing or inhibiting the activity of extracellular lipase, extracted from *P.aeruginosa*. While some had negligible effect on lipase; iron, zinc, calcium and copper reduced the enzyme activity, whereas mercury strongly inhibited it. A metal cofactor may not be typically involved in the mechanism of lipase catalysis, but number of reports has demonstrated many divalent cations stimulate or inhibit its activity[12;13](El Khattabi et al, 2003; Lu et al, 2013). Many studies done on lipasesrevealed that metal ions in number of ways are responsible for the maintenance of the stability of lipase[14;15;16]. In one of the study done on lipase from *Bacillus* shows that magnesium, iron, manganese and cobalt enhance the effect of enzyme when taken in 1mM concentration and sodium, EDTA and SDS show significantly inhibitory effect[9]. Our study also demonstrates similar enhancing effects of metal cobalt on extracellular lipase enzyme. Aysun and Alper[16]have tried to find out whether the different metal ions stabilize or destabilize the enzyme isolated from *Pseudomonas aeroginosa*, and they found that $Ca^{2+}$ and $Ni^{2+}$ stimulated the enzyme activity only by 3.5% to 9.5% respectively and not much decrease was observed in the presence of $Mn^{2+}$,$K^{2+}$,$Mg^{2+}$ and $Na^{2+}$ as the enzyme retained more than 83% of its activity.Another study done by Tiwariet al[17]on thermo tolerant lipase reported that a concentration of 50µg/ml of iron, manganese, zinc, cobalt, copper and mercury ions showed above 60-70% relative lipase activity; whereas barium, calcium, magnesium enhanced the activity[18].

The potential of lipases in food and other industries shows the need to develop novel cost-effective technologies for increased production, scaling up and purification of this versatile enzyme. Other than the food industry, lipases have been applied in the synthesis of fine chemicals, the production of biopolymeric materials, biodiesel production, detergent industry, textile industry, paper and pulp industry, the synthesis of ingredients for personal care products, the synthesis of surfactants and of structural triglycerides, agrochemical production, the oleochemical business, the pesticide industry and in environmental management. The present study demonstrated the inhibitory as well as enhancing effects of metals on lipase enzyme, extracted from *Pseudomonas aeruginosa*. Though more exhaustive work is still needed, such studies may help in optimizing the growth conditions for scaling up enzyme production.

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