

## Influence of Metal Ions on Activity and Stability of $\alpha$ -amylase from *Bacillus licheniformis* ATCC 6346

Vengadaramana, A., Balakumar, S. and Vasanthi, A.

Effect of different metal ions on the activity and stability of  $\alpha$ -amylase produced by *Bacillus licheniformis* ATCC 6346 was investigated. Here 2mM of  $Mn^{2+}$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $Hg^{2+}$ ,  $Mg^{2+}$ ,  $Ba^{2+}$  and  $Cu^{2+}$  and 0.5mM Ethylenediaminetetra-acetic acid were used. Before commencing the studies the enzyme from the spent medium was precipitated with 50% ammonium sulphate and dialyzed against distilled water. This dialysis was carried out at 20°C for 48h.  $\alpha$ -Amylase activity was strongly inhibited by 2mM  $Cu^{2+}$ ,  $Hg^{2+}$ ,  $Mn^{2+}$  and 0.5mM Ethylenediaminetetra-acetic acid but less affected by 2mM  $Mg^{2+}$  and  $Ba^{2+}$ . 2mM  $Ca^{2+}$  and  $Na^+$  stimulated the enzyme activity at 85°C and at pH 7.0. Effect of NaCl on the stability of  $\alpha$ -amylase was studied. The enzyme was pre incubated with different concentrations of NaCl (0 to 0.4M) and 21% and 1.0% of initial activities were retained with 0.4M and without NaCl respectively at 60min of pre-incubation at 85°C and pH 7.0. However maximum activity was retained with 0.1M NaCl (33% of initial activity) at 60min incubation at 85°C and pH 7.0. In 0.1M NaCl 100% of initial enzyme activity was retained for 150min and 70min of pre-incubation at 60°C and 70°C respectively and at 80°C, 88.20% of its initial activity was retained at 60min of pre-incubation at pH 7.0. The effect of  $Ca^{2+}$  on the stability of the enzyme was studied. The enzyme was pre-incubated with different concentration of  $Ca^{2+}$  (0 to 1mM). In presence of 1mM  $Ca^{2+}$ , 100% of initial activity was retained at 60min of pre-incubation at 85°C and at pH 7.0. The effect of  $Ca^{2+}$  and  $Na^+$  combination on the stability of  $\alpha$ -amylase was studied. With 1mM  $Ca^{2+}$  and 0.1M NaCl, 17.3% of its initial activity was retained at 180min of pre incubation at 95°C and at pH 7.0 but the enzyme with 1mM  $Ca^{2+}$  and 0.1M NaCl separately, lost total activity at 120 and 90min respectively. Protein denaturants, such as Sodiumdodecylsulphate (10mM), decreased the enzyme activity; in contrast, urea (10mM) had no influence on enzyme activity. The enzyme in 0.1 and 0.5M NaCl showed 104 and 74.7% of the original activity respectively at 24h of incubation at 6°C and pH 7.0.