Thermostable alkaline phytase from *Bacillus* sp. MD2: Effect of divalent metals on activity and stability

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Abstract

Phytate, the major source of phosphorus in seeds, exists as a complex with different metal ions. Alkaline phytases are known to dephosphorylate phytate complexed with calcium ions in contrast to acid phytases that act only on phytic acid. A recombinant alkaline phytase from *Bacillus* sp. MD2 has been purified and characterized with respect to the effect of divalent metal ions on the enzyme activity and stability. The presence of Ca^{2+} on both the enzyme and the substrate is required for optimal activity and stability. Replacing Ca^{2+} with Ba^{2+}, Mn^{2+}, Mg^{2+} and Sr^{2+} in the phytase resulted in the expression of > 90% of the maximal activity with calcium-phytate as the substrate, while Fe^{2+} and Zn^{2+} rendered the enzyme inactive. On the other hand, the calcium loaded phytase showed significant activity (60%) with sodium phytate and lower activity (17–20%) with phytate complexed with only Mg^{2+}, Sn^{2+} and Sr^{2+}, respectively. On replacing Ca^{2+} on both the enzyme and the substrate with other metal ions, about 20% of the maximal phytase activity was obtained only with Mg^{2+} and Sr^{2+}, respectively. Only Ca^{2+} resulted in a marked increase in the melting temperature (T_m) of the enzyme by 12–21 °C, while Ba^{2+}, Mn^{2+}, Sr^{2+} or Cu^{2+} resulted in a modest (2–3.5 °C) increase in T_m. In the presence of 1–5 mM Ca^{2+}, the optimum temperature of the phytase activity was increased from 40 °C to 70 °C, while optimum pH of the enzyme shifted by 0.4–1 pH unit towards the acidic region.