Thermostable alkaline phytase from Bacillus sp. MD2: Effect of divalent metals on activity and stability. Author/s Tran Thi Thuy, Suhaila Hashim, Yasser Gaber, Gashaw Mamo, Bo Mattiasson, Rajni Hatti-Kaul Department/s Biotechnology (LTH)

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Abstract English 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 1-5mM 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. Subject Chemistry

Keywords Alkaline phytase, Phytate, Divalent metal ions, Differential scanning, calorimetry
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