

Current biochemical research on phytase genes in micro-organisms and plants

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The stepwise release of phosphate from phytate, the major storage form of phosphate in plant seeds and pollen, is initiated by a class of enzymes that have been collectively called phytases. The classification is solely due to the *in vitro* capability of these enzymes to accept phytate as a substrate. Phytase is an inducible enzyme in most micro-organisms and its expression is subjected to a complex regulation, but phytase formation is not controlled uniformly among different micro-organisms. Until now, phytase production was studied in some detail only in *Escherichia coli* (Touati *et al.*, 1987; Greiner *et al.*, 1993), *Raoultella terrigena* (Greiner *et al.*, 1997; Zamudio *et al.*, 2001) and *Saccharomyces cerevisiae* (Andlid *et al.*, 2004). In moulds, phytase production is growth associated; enzyme activity increases from the onset of growth to the beginning of the stationary phase. However, in non-limiting media the formation of many bacterial phytases halts in exponentially growing cells and begins when cultures enter the stationary phase. This suggests that either nutrient or energy limitation, known to occur in the stationary phase, could be responsible for phytase induction.

Among the nutrient limitations tested, only carbon starvation provoked immediate synthesis of the *R. terrigena* phytase, whereas in *Bacillus* and *E. coli*, phytase synthesis was triggered when bacteria were starved of inorganic phosphate, while carbon, nitrogen, and sulfur limitation were ineffective. A tight regulatory inhibition of phytase formation by phosphate concentration was generally observed in all microbial phytase producers, including moulds, yeast, and bacteria with the exception of *R. terrigena* and the rumen bacteria. Thus, phytase synthesis is regulated at transcription level by derepression. The repression of phytase synthesis by inorganic phosphate seems to be less significant in more complex media, although it is not known which components account for the reduced repression.

In addition, phytase expression depends on medium pH and the nature of the C source used for growth (Konietzny and Greiner, 2004). In the presence of simple sugars, strong repression of enzyme synthesis was observed. In moulds, formation of mycelial pellets was responsible for the low enzyme yields. That cAMP-CAP, rather than the C source itself, are directly involved in the regulation of phytase synthesis was shown in *E. coli*. Synthesis of the phytase in both *E. coli* and *R. terrigena* were reported to be negatively regulated by cAMP. For several *Raoultella* sp. it was shown that phytate is needed to induce phytase production. Substrate induction was also found in *Mitsuokella jalaludinii*, whereas phytate had no effect on the formation of phytase in *Escherichia coli*. Phytase formation in *Pseudomonas* sp. and *R. aerogenes* was reported to be significantly induced in the presence of *myo*-inositol as the sole carbon source, but not in other *Raoultella* sp.

The efficient induction or derepression of phytase formation by phosphate starvation in most micro-organisms raises the question of a possible role in providing the cell with phosphate hydrolysed from molecules such as phytate. This hypothesis is supported by the identification of a phytase in the stalk of *Caulobacter crescentus*, a gram-negative alpha-purple proteobacterium, which is an oligotroph that lives in low-nutrient aquatic environments. Phosphate is the limiting nutrient in the environments where *C. crescentus* is found, and one of the hypothesised functions of the stalk is phosphate uptake. Stalks elongate when phosphate is limiting, increasing the surface area available for phosphate

uptake; the presence of a phytase also would allow the uptake of the organically phosphate by the stalk (Ireland *et al.*, 2002). In addition, ruminants seem to digest phytate through the action of phytase produced by microbes in the rumen. In contrast to most other bacteria, anaerobic rumen bacteria can tolerate a high level of phosphate without any negative impact on phytase production. This unique ability leads to a more efficient phytate hydrolysis in the rumen, even under the high phosphate levels in the rumen fluid of ruminants fed concentrated feed. The phosphate generated by splitting of phytate is utilized by both the microbial flora and ruminant host.

It was suggested that soil micro-organisms colonising the plant rhizosphere and producing extracellular phytase activity, such as *Bacillus* and *Enterobacter* ssp. as well as mycorrhizal fungi, could promote plant growth by making phytate available to the plant. In addition, phytate-degrading enzymes have been suggested to play a role in inositol phosphate metabolism or perhaps microbial pathogenesis. Research into the mechanisms by which pathogens cause disease implicated acid phosphatases and inositol phosphate phosphatases as effectors that help to undermine host cell signalling and defence mechanisms. In particular, inositol phosphate phosphatases from some pathogenic members of the Enterobacteriaceae have been shown to be important virulence factors after they are injected into the host cell by Type III secretion systems. Once inside the host cell, these phosphatases have the potential to interfere with a variety of host cell signalling pathways, including those affecting cellular survival and regulation of intracellular membrane trafficking (DeVinney *et al.*, 2000).

In higher plants, phytases occur predominantly in grains, seeds and pollen (Konietzny and Greiner, 2002), where they are responsible for phytate degradation during germination to make phosphate, minerals, and *myo*-inositol available for plant growth. Grains, seeds and pollen contain both constitutive and germination-inducible phytases. Although large increases in phytase activities occur in germinating seeds as well as in germinating pollen, the biochemical mechanism leading to this rise in phytase activity is not well understood. In pollen, phytases induced during germination may be synthesised from long-lived, pre-existing mRNA. In cereals and legumes, the cause of the rise in phytate-degrading activity during germination is also disputed. Some studies suggest *de novo* synthesis, whereas others suggest simple activation of pre-existing enzymes. During germination phytase activity may be controlled by the action of gibberellic acid and phosphate. Recently, it was shown that gibberellic acid does not stimulate phytase activity in an *in vitro* assay using a phytase from lentils and that gibberellic acid does at least partly stimulate *de novo* synthesis of the lentil phytase(s) during germination. Two main mechanisms appear to be involved in the regulation of phytase activity by phosphate. Acid phytases are strongly inhibited by phosphate, thus the enzyme activity itself may be controlled by phosphate. It was concluded that phosphate also acts at the transcription level, since phosphate added early enough in the germination sequence can repress the increase in phytase activity.

References

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