Unique alkaline phytase from lily pollen: cloning, characterization and differential expression

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Phytases catalyze the hydrolysis of phytic acid (*myo*-inositol hexa*kis*phosphate), the most abundant inositol phosphate in cells. High concentration of phytic acid in animal feed leads to environmental and nutritional problems. Recent investigations have indicated that animal feed supplemented with phytase alleviates both nutritional and environmental problems. Therefore, phytases have become commercially important enzymes as feed and food supplements.

Although acid phytases from fungal sources have been extensively studied, there is a paucity of information regarding alkaline phytases. Alkaline phytases with unique biochemical and structural properties have been identified in plants and bacteria. The alkaline pH and thermal stability of the enzyme suggests that these enzymes may be good candidates as animal feed supplements. The lack of structural and mechanistic information about alkaline phytase from plant tissues is partially due to the challenges in purifying phytases from plants including low endogenous levels, low stability and contamination from non-specific phosphatases. The availability of cDNA expression clones of alkaline phytase will allow us to gain knowledge on the structural, molecular and mechanistic aspects of enzymes. Purification of an alkaline phytase from the pollen of *Lilium longiflorum* was achieved in our laboratory by a multi-step procedure. The cloning and characterization of alkaline phytase cDNA from lily pollen will be described.

Six internal peptides from purified lily pollen alkaline phytase were sequenced by a combination of Edman degradation and electrospray ionization-tandem mass spectroscopy methods. The amino acid sequence data was used to design degenerate oligonucleotide primers. The complete cDNA sequence of alkaline phytase was obtained by using a combination of reverse transcriptase–polymerase chain reaction (RT-PCR), 5' "rapid amplification of cDNA ends" (RACE) and 3' RACE techniques. Three isoforms of alkaline phytase transcripts from lily pollen were sequenced. *LlAlp1*, *LlAlp2*, and *LlAlp3* encodes for peptides with 487, 408, and 511 amino acids, respectively. All the isoforms possess the conserved active site motifs, 'RHGXRXP' and 'HD', characteristic of histidine acid phosphatases. However, lily pollen alkaline phytase shows low identity (<25%) to all known phytases including fungal histidine acid phosphatases and multiple inositol polyphosphate phosphatases. Phylogenetic analysis of histidine phosphatases revealed that the histidine phosphatase family of enzymes can be divided into four subgroups. *LlAlp1*, *LlAlp2* and *LlAlp3* are more closely related to the multiple inositol polyphosphate phosphatase containing subgroup rather than the maize and fungal acid phytases.

The expression level of the three isoforms was investigated in leaves, stem, petals and pollen of *Lilium longiflorum* using semi-quantitative reverse-polymerase chain reaction. These studies suggest that the LlALP gene is expressed in all tissues investigated however, differential expression of the three isoforms was observed. During the early stages of flower development expression of one isoform was observed however, all three isoforms were

expressed at the later stages. Maximum expression of all three isoforms was found in mature pollen grains.

This is the first report of the cloning of alkaline phytase cDNAs from plants. Availability of the cDNA clones will allow us to further our understanding of the molecular characteristics, structure activity relationships and physiological role of alkaline phytases.