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In silico analysis and comparison of phytase gene from *Aspergillus niger*

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Abstract

Phytase gene releases extracellular phytase which degrades phytate to release phosphorus. In plants, phosphorus is primarily stored as the phytate..Expressing phytase gene to upgrade the production of phosphors is common these days. The most commonly used source of phytase gene is Aspergillus niger. To properly use phytase gene for transgenic purposes, in silico analysis is crucial to know important aspects and parameter of gene. In this study, in silico analysis and comparison of Aspergillus niger phytase gene was done using different bioinformatics approaches to identify the phytase gene.

Keywords: Silico analysis, *Aspergillus niger*, Phytase gene.

Introduction

Phytase hydrolyzes phytate or phytic acid which is an organic form of phosphorus and release utilizable inorganic phosphorus. In grains and oil seeds, it exists in non-digestible form[1]. Plants, animals and bacteria have phytases in them. However, fungi phytases are well characterized and almost every phytasehas been detected in them[2]. Plant seeds contain meager amount of phytate but monogastric animals are unable to digest that phytate. Scientists have been trying to enhance phytate –P bioavailability in animal feed. They are focusing on the overexpression of phytase genes in plant seeds[3]. The enzyme is being produced by fermentation technology but the cost of production is very high. If scientists become successful in the transgenic production of high phytase activity in plant seedsthen expenses of fermentation technology[4]. Phytase gene has been over expressed in wheat grains[5], soybean seeds[6,7], canola seeds[8,9] and maize grains[10] and it was found that phytase activity was clearly high.

Phytate contains an inositol surrounded by six phosphate ester bonds. The negative charge on the molecule is attributed by phosphate bonds. It acts as a chelating agent and reacts with the minerals like Ca^{2+}, Zn^{2+} and Fe^{2+} and form complexes. These complexes decrease the availability of minerals and amino acids[11].

It has been reported that roots and root exudates of plants contain phytases[12-14]. But this exudate phytase do not carry out the proper assimilation of organic phosphorus for plants[3,14]. When exogenous phytase was added into the medium, plants did utilization ofphytate[13,15,16]. Scientists have over expressed phytase in plants roots to enhance organic phosphorus uptake. In one study, over expression of *phyA* in transgenic *Arabidopsis* with the *Pht1;2* promoter resulted in improved usage and assimilation of phosphorus and phytase activity. In this study, transgenic plants were able to grow in a medium when phytate as the sole phosphorus source was provided[17,18].

In the present study, in-silico analysis of *Aspergillus niger* phytase gene was done.

Materials and Methods

Bioinformatics approach for sequence analysis: Sequence analysis of phytase gene was done through NCBI web portal. After adding the accession number of gene ACE79229 along with the selection of nucleotide option, sequence of phytase gene of size 1.4kb was attained[19].

Domain Analysis: Domain analysis was done using CDD (conserved domain database)[20].

Phylogenetic analysis of phytase gene: Matching of query sequence of phytase gene was done with other species through SEQR search in NCBI[21].

Level of identity: Identity matching of *Aspergillus niger* phytase gene with other gene sequences was obtained through SEQR-NCBI[22].

Protein Domain analysis: Protein domains were analyzed using pfam to show the domains of different protein families found in the gene sequence. The location of the coding region was obtained from the protein domain analysis[23].

Results and Discussion

Phytase gene sequence retrieval: Definition: *Aspergillus niger* strain BCC18081 PhyA (phyA) mRNA.
Accession: EU786167.

ATGGGCGTCTCTGCTGTTCTACTTCCTTTGTATCTCCTGTCTGGAGTCACCTCCGGACTGGCAGTCCCCGC**CTCGAGA**
AATCAATCCACTTGCGATACGGTCGATCAGGGGTATCAATGCTTCTCCGAGACTTCGCATCTTTGGGGTCAAT
ACGTGCCGTTCTTTTCTCTGGCAAACGAATCGGCCATCTCTCCTGATGTGCCCGCCGGATGCCGAGTCACTTT
CGCTCAGGTCCTCTCCCGTCATGGAGCACGGTATCCGACCGACTCCAAAGGCAAGAAATACTCCGCTCTCATT
GAGGAGATCCAGCAGAATGCGACCACCTTTGATGGGAAATATGCCTTCCTGAAGACATACAACTACAGCCTG
GGTGCAGATGACCTGACTCCTTTCGGAGAACAGGAGCTAGTCAACTCCGGCATCAAGTTCTATCAGCGATACG
AATCGCTCACAAGAAACATCATTCCATTCATCCGATCCTCTGGCTCCAGCCGCGTGATCGCCTCCGGCAAGAAATTC
ATCGAGGGCTTCCAGAGCACCAAGCTGAAGGATCCTCGTGCCCAGCCCGGCCAATCGTCGCCCAAGATCGACGTGGT
CATTTCCGAGGCCAGCTCATCCAACAACACTCTCGACCCAGGCACCTGCACCGTCTTCGAAGACAGCGAATTGGCCG
ATGCCGTCGAAGCCAATTTCACCGCCACGTTCGTCCCCACCATTCGTCAACGTCTGGAGAACGACCTGTCTGGCGTGT
CTCTCACAGACACAGAGGTGACCTACCTCATGGACATGTGCTCCTTCGACACCATCTCCACCAGCACCGTCGACACC
AAGCTGTCCCCCTTCTGTGACCTGTTCACTCATGACGAATGGATCAACTACGACTACCTCCAGTCCCTGAAAAAGTAC
TACGGCCATGGCGCGGGTAACCCGCTCGGCCCGACCCAGGGCGTCGGCTACGCTAACGAGCTCATCGCCCGTCTCAC
CCACTCGCCTGTCCACGATGACACCAGCTCCAACCACACATTGGACTCTAACTCGGCTACCTTTCCGCTCAACTCTAC
TCTCTACGCGGACTTTTCCCACGATAACGGCATCATCTCTATTCTCTTTGCTTTGGGTCTGTATAACGGCACCAAGCC
GCTGTCTACCACGACCGTGCAGAATATCACCCAGACAGATGGATTCTCGTCTGCTTGGACGGTTCCGTTTGCTTCGCG
TCTGTACGTCGAGATGATGCAATGTCAGGCGGAGCAGGAGCCGCTGGTCCGTGTCTTGGTTAATGATCGCGTTGTCC
CGCTGCATGGGTGTCCGGTTGATGCTTTAGGGAGATGTACCCGGGATAGCTTTGTGAAGGGGTTGAGCTTTGCTAGA
TCTGGGGGTGATTGGGCGGAGTGTTTTGCTTAG

Figure-1
The highlighted region is the coding sequence (Exon)

Domain analysis: Histidine phosphatase domain was found in phytase gene (Figure-2).

Phylogenetic study: The query sequence was most similar to 3-phytase A of *Aspergillus niger* CBS 513.88 (Figure-3).

Identity Matching: As in Figure-4, it was found that 3-phytase A of Accession number XP_001401713 was 93% identical to the query sequence while phytase of Accession number EEA22654 was least identical to the query sequence.

Protein domain analysis: His Phos 2 was found in pfam analysis (Figure-5).

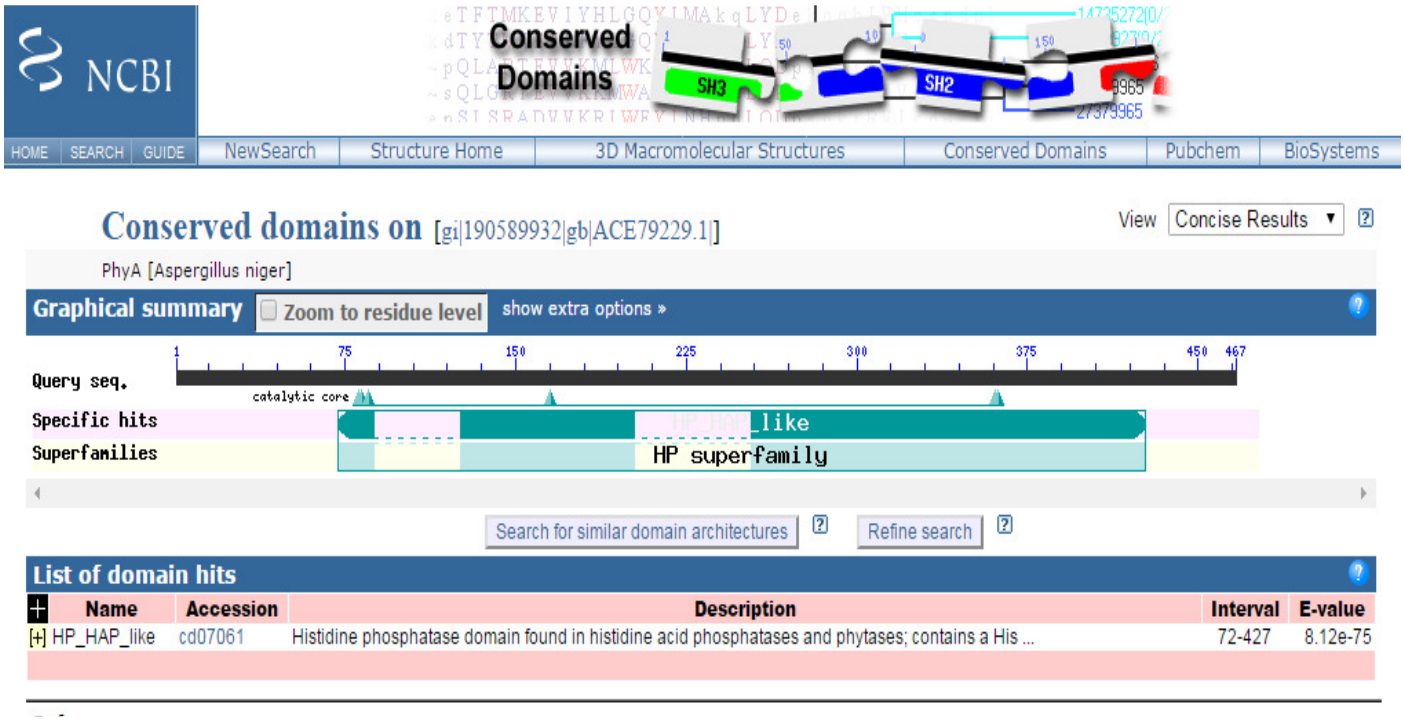

Figure-2
Histidine phosphatase domain was found in phytase gene

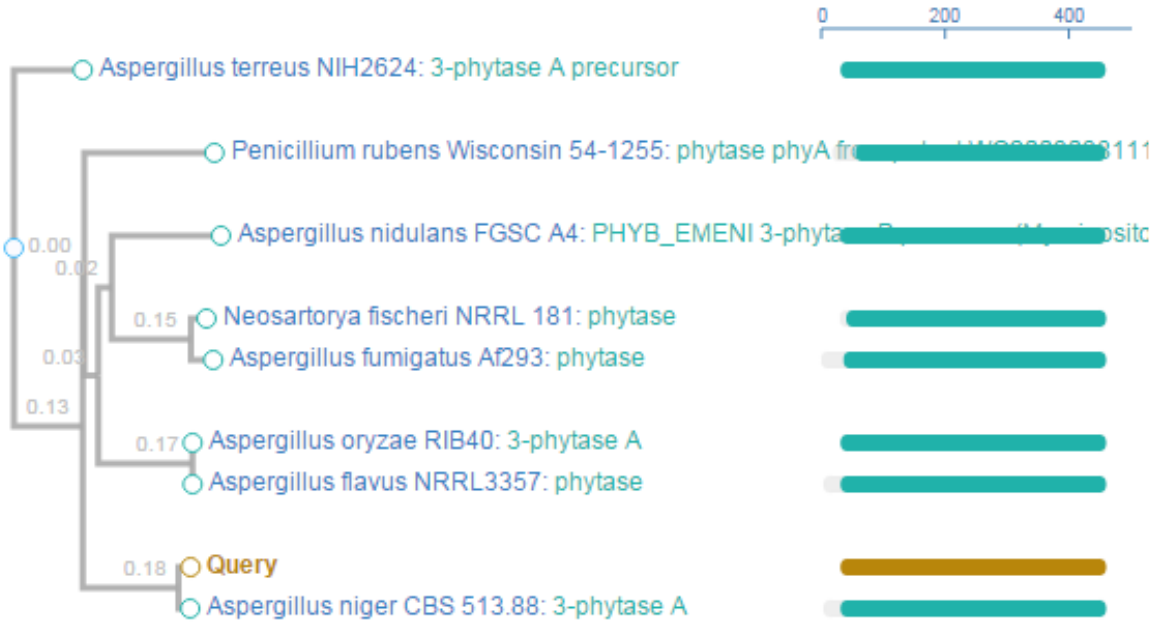

Figure-3
The query sequence was most similar to 3-phytase A of *Aspergillus niger* CBS 513.88

	Name	Identity
☐	XP_001401713 : 3-phytase A	93%
☐	Q9C1T1 : 3-phytase A	60%
☐	EAW25263 : phytase	60%
☐	EED53727 : phytase	59%
☐	CAP93440 : phytase phyA from patent WO2003038111-A2-Penicillium chrysogenum	59%
☐	EAL89926 : phytase	58%
☐	EAA64805 : PHYB_EMENI 3-phytase B precursor (Myo-inositol-hexaphosphate 3-phosphohydrolase B) (3 phytase B) (Myo-inositol hexakisphosphate phosphohydrolase B)	56%
☐	EAU34402 : 3-phytase A precursor	55%
☐	EAW10331 : phytase	51%
☐	EEA22654 : phytase	51%

Figure-4
Identity Matching

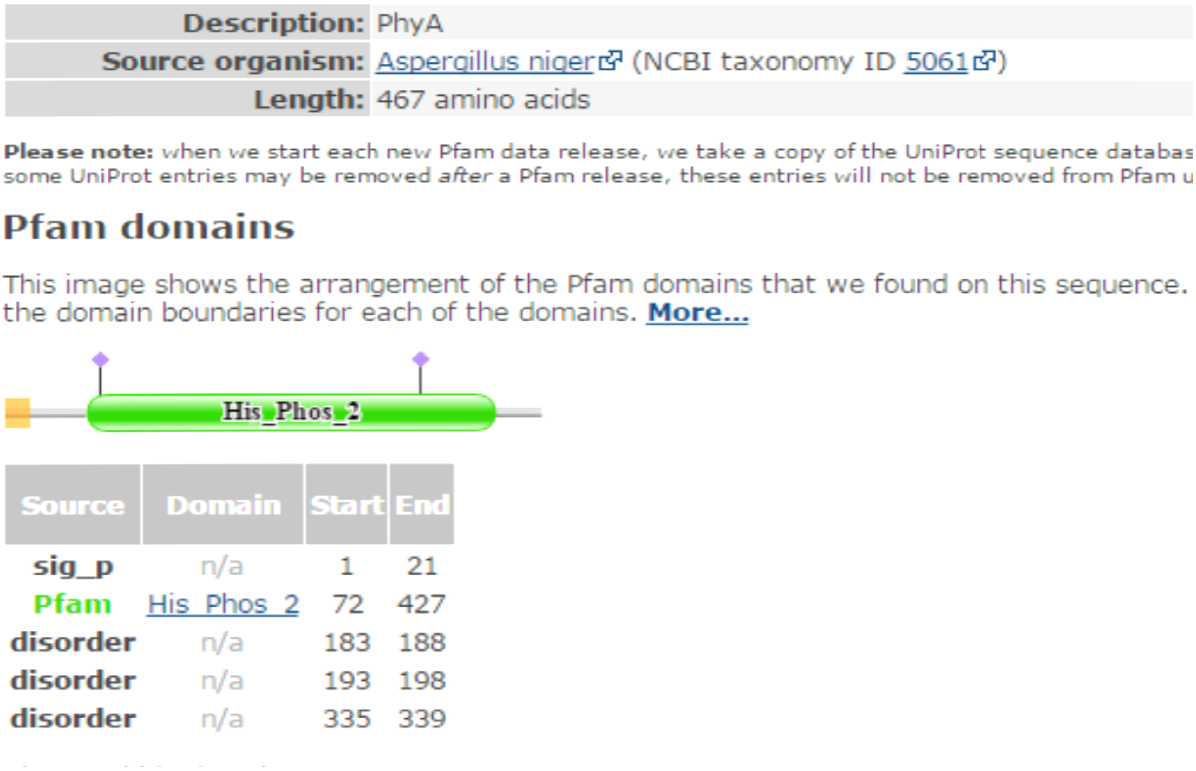

Figure-5
His Phos 2 was found in pfam analysis

Discussion: Phosphorus is the plant nutrient which is least available in soil as plants utilizes only soluble inorganic form of phosphorus[24]. There is an important class of phosphatases called phytases (InsP6 phosphohydrolase). They carry out the sequential hydrolysis of phytic acid or phytate and release less phosphorylated myo-inositol derivatives and inorganic phosphate[3]. Phytase gene releases extracellular phytase which degrades phytate. This phytate is decomposed to release phosphorus and hence increases the phosphorus uptake for plants. Because of its nutritional importance, phytase is under the limelight for a long time. Overexpression and production of phytase transgene is a promising way to increase phytase activity. By implying various bioinformatics tools biochemical features, homology search, multiple sequence alignment, phylogenetic tree construction, motif, and superfamily distribution of proteases can be investigated. Specific degenerate primers are used for identification purposes. These primes can be designed by using Conserved sequences in motifs. This can also be used for the isolation of type and class of phytases[25].

Conclusion

By using bioinformatics tools it's easy to do phylogenetic analysis and one can investigate variation among sequences. These types of analytical tools can reveal further information about different phytases and further classification of highly diverse HAPhys. This information can further tell about their selection for various application purposes. Analysis and identification of genes through various bioinformatics software's is useful to understand the gene expression studies of phytase gene. Computational analysis through different bioinformatics approaches might be used for future genetic engineering of the diverse and important class of selected phytase gene and can be useful in future characterization of phytase gene.

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