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**International Journal of Pharmaceutical Research & Allied Sciences, 2016, 5(3):1-7**


**Research Article**

**ISSN : 2277-3657**
**CODEN(USA) : IJPRPM**

# Comparative Study of *Phytase* Enzyme from Plants and Microbes using Bioinformatic Tools

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**ABSTRACT**

*The food industry plays an important role in our present day society. Enzymes, proteins, vitamins and several other factors contribute a lot to the food industry. Consuming healthy food is so necessary in order to prevent various diseases. The total amount of nutrients, levels of antinutritive factors determines the nutritional quality of the food consumed. One such anti-nutritional factor, 'phytic acid' (myoinositolhexakis-dihydrogenphosphate), is a major storage form of organic phosphorous in legumes, cereals, oilseeds and nuts. Due to its chelation of various cations, in humans, reduction in the digestibility of proteins, starch and lipids occurs. The enzyme 'phytase' (myoinositolhexaphosphatephosphohydrolase) belonging to acid phosphatases, have the capacity to hydrolyze phytic acid to a series of lower phosphate esters of myoinositol and phosphate. The phytase enzyme contribute to food industry by decreasing the levels of phyticacid in the food and making the food safer for consuming. In plants,the enzyme is produced by various cereals and legumes and from microbes such as Aerobacter, Bacillus, Klebsiella sp. In the present study, insilico work in phytase enzyme has been done from both plants and microbes using various bioinformatic tools as it is not much explored. Initially, the primary sequence of the phytase enzyme from plant source Vignaradiata and from microbe Bacillus subtilis was obtained from Gen Bank at National Centre for Biotechnology Information (NCBI). Sequence analysis of the phytase enzyme was done using Basic Local Alignment Search Tool (BLAST)with five randomly selected plant species and microbial species. With this primary sequence, the different physico-chemical properties, identification of motifs and domains, functional analysis and Multiple Sequence Alignment (MSA) of the phytase enzyme were studied. In this individual study of phytase from plants and microbes, all these properties revealed that the enzyme phytase share 70 to 100 percentage of similarity in the physico-chemical properties and plays a very major role in hydrolysis of phytic acid thereby decreasing its toxicity. Thus, phytase from these plants and microbes can be recommended to food industry as a nutritive factor to increase the food quality by decreasing the levels of phytic acid.*

**Keywords***: Phytase*, phytic acid, BLAST, MSA, food industry, nutritive enzyme.

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## INTRODUCTION

Food plays an important role in human health aspects. Enzymes, proteins, vitamins and several other factors contribute a lot to the food industry. Consuming healthy food is of great importance in our present day society. The amount of nutrients, levels of antinutritive factors differs in various kinds of foods. One such anti-nutritional factor, 'phytic acid' (myoinositolhexakis-dihydrogenphosphate), is a major storage form of organic phosphorous. It isfound

in legumes, cereals, oilseeds and nuts [5]. Accumulation of phytic acid in cereals and legumes is found in aleurone particles and globoid crystals [8]. Due to its chelation of various cations, in humans, reduction in the digestibility of proteins, starch and lipids occurs. Phytic acid binds different mono-, di-, and trivalent cations and their mixtures, resulting in formation of insoluble complexes [8]. The formation of insoluble phytatemineral complexes in the intestinal tract prevents mineral absorption. This reduces the bioavailability of essential minerals [1]. Phytic acid also complexes with the proteins, amino acids and make them less soluble inhibiting the digestive enzymes [6].

Hydrolysis of phytic acid can be carried out through enzymatic treatment. One such enzyme 'phytase' (myoinositolhexaphosphatephosphohydrolase) belonging to acid phosphatases, have the capacity to hydrolyze phytic acid to a series of lower phosphate esters of myoinositol and phosphate. The phytase enzyme contribute to food industry by decreasing the levels of phytic acid in the food and making the food safer for consuming. Phyases are widespread in nature occurring both in plants and microbes. The various plant products produce phytase which take part in hydrolysis of phytic acid.In plants, the enzyme is produced by various cereals and legumes and from microbes such as *Aerobacter*, *Bacillus*, *Klebsiella sp*. Increase in phytic acid levels can be decreased through phytase enzyme. Therefore, phytase has become an important industrial enzyme. The milestones in discovery and commercialization of phytase were also described which leads to analysis of phytases[10].

In addition to all these, phytase would be an environmentally friendly product, thereby reducing the amount of phosphorous entering into the environment.

Bioinformatics plays an important role in analysis of various enzymes, proteins, vitamins. Various kinds of databases and softwares are available which are being used for analyzing the phytase enzyme. Simultaneously various kinds of biological properties can be analysed within a short period of time using these various bioinformatic tools. The present study deals with the phytase enzyme produced from plants and microbes. The primary sequence was obtained from Gen Bank at National Centre for Biotechnology Information (NCBI). Sequence analysis was done using Basic Local Alignment Search Tool (BLAST) and the following five different plants- *Vigna radiata*(Green gram), *Glycine soja*(Wild soybean), *Medica gotruncatula*(Barrel clover), *Cicera rietinum*(Chick pea), *Theobroma cacao*(Cocoa)and five different species of *Bacillus- Bacillus subtilis, B.mojavensis, B.amyloliquefaciens, B.methylotrophicus* and *B.siamensis* were randomly selected based on similarity percentage. The test species among the selected plants and microbes were *Vigna radiata* and *Bacillus subtilis*.

## MATERIALS AND METHODS

### Sequence retrieval

The primary sequence of the phytase enzyme from plant (*Vigna radiata*, Acc. No. ABW76419.1) and microbe (*Bacillus subtilis*, Acc. No. WP_019258689.1) was retrieved from the Gen Bank [4] at National Centre for Biotechnology Information (NCBI) [7].

### Homology search

BLAST-P analysis was carried out (blast@ncbi.nlm.nih.gov). Based on similarity percentage, five sequences of phytase enzyme produced from different plants and microbes were selected randomly and used for further analysis of various other properties and parameters responsible for the hydrolysis of phytic acid thereby decreasing the levels of phytate.

### Analysis of physico-chemical properties

Using the various sequences of phytase from plants and microbes, the physico-chemical properties were studied with the help of the tool ExPASyProtParam server (http://expasy.org/cgi-bin/protparam). Physico-chemical properties like number of amino acids, molecular weight (M.Wt), Isoelectric point (pI), Extinction coefficient (EC), Aliphatic index (AI), Grand Average Hydropathy (GRAVY) of the sequences were analysed.

### Identification of Domains

Conserved Domain Database (CDD) was used to identify the domains of the protein. It is a resource collection of various ancient domains. The identification is of great importance in order to know the parameters responsible for hydrolysis of phytic acid.

**Multiple Sequence Alignment (MSA)**
Multiple Sequence Alignment (MSA) was carried out with the phytase enzyme sequences from plants and microbes using a tool, Clustal Omega.

**Phylogenetic Analysis**
The ancestral analysis among the various randomly selected plants and microbes producing phytase enzyme was worked out through phylogenetic analysis through Neighbour Joining (NJ) method. The cladogram was obtained and the phylogenetic relationship among the organisms were studied.The analysis leads to the study of closeness among the phytase enzyme produced from various plants and microbes selected.

## RESULTS AND DISCUSSION

**Sequence retrieval and homology search**
The primary sequence of phytase produced by *Vigna radiata* and *Bacillus subtilis* retrieved from GenBank, in the FASTA format were analyzed for homology using BLAST-P. In the BLAST-P result page, the sequences were arranged in descending order of identity percentage. From the sequences, based on similarity percentage, five sequences from plants (Table.1) and five sequences from microbes (Table.2) were obtained.

**Table.1 Five plant sequences of phytase selected from BLAST-P**

| S.No. | Description | Ident. | Accession Number |
|---|---|---|---|
| 1 | *Vigna radiata* | 100% | ABW76419.1 |
| 2 | *Glycine soja* | 92% | KHN48791.1 |
| 3 | *Medicago truncatula* | 88% | XP_003618565.2 |
| 4 | *Cicer arietinum* | 83% | XP_004489427.1 |
| 5 | *Theobroma cacao* | 80% | XP_007034462.1 |

**Table.2 Five microbial sequences of phytase selected from BLAST-P**

| S.No. | Description | Ident. | Accession Number |
|---|---|---|---|
| 1 | *Bacillus subtilis* | 100% | WP_019258689.1 |
| 2 | *B.mojavensis* | 86% | WP_024121774.1 |
| 3 | *B.amyloliquefaciens* | 71% | WP_045511202.1 |
| 4 | *B.methylotrophicus* | 70% | WP_046702299.1 |
| 5 | *B.siamensis* | 70% | WP_045926145.1 |

The similarity search for the plant sequences carried out with the help of BLAST tool indicated 92% similarity to the second sequence, phytase (*Glycine soja*) and the similarity search for the microbial sequences carried out indicated 86% similarity to the second sequence, phytase (*Bacillus mojavensis*).

**Analysis of physico-chemical properties**
The ProtParam, ExPASy tool server exhibited the physico-chemical properties of the phytase from plants and microbes. In *Vigna radiata*,there are 287 amino acids in the sequence and *Bacillus subtilis* with 382 amino acids. The molecular weight (M.Wt) was 31989.8for *Vigna radiata* and 41746.1 for *Bacillus subtilis* and the theoretical pI value (pI) was 5.11 and 4.91for *Vigna radiata* and *Bacillus subtilis*. The extinction coefficients (EC) was found to be 77935 M◌-1 cm-1 and43320 M◌-1 cm-1,followed byaliphatic index (AI) with the value of 68.61and73.53. Then, the Grand Average of Hydropathicity (GRAVY) in the protein was -0.313 and -0.568of *Vigna radiata* and *Bacillus subtilis* respectively. The physico-chemical properties for all the plant and microbial sequences are given below(Table.3 and 4). The tool was not able to compute the molecular weight and pI value of *Cicer arietinum* sequence as it contained several consecutive undefined amino acids.

**Table.3 Physicochemical properties of phytase plant sequences computed using Protparam tool**

| S. NO. | DESCRIPTION | ACCESSION NUMBER | NUMBER OF AMINO ACIDS | M.WT | pI | EC | AI | GRAVY |
|---|---|---|---|---|---|---|---|---|
| 1. | *Vigna radiata* | ABW76419.1 | 287 | 31989.8 | 5.11 | 77935$M^{-1}cm^{-1}$ | 68.61 | -0.313 |
| 2. | *Glycine soja* | KHN48791.1 | 656 | 73773.6 | 6.13 | 145830$M^{-1}cm^{-1}$ | 78.11 | -0.205 |
| 3. | *Medicago truncatula* | XP_003618565.2 | 587 | 66165.0 | 6.14 | 114305$M^{-1}cm^{-1}$ | 76.05 | -0.292 |
| 4. | *Cicerarietinum* | XP_004489427.1 | 587 | - | - | 129775$M^{-1}cm^{-1}$ | 72.73 | -0.278 |
| 5. | *Theobroma cacao* | XP_007034462.1 | 499 | 55747.1 | 5.16 | 97345$M^{-1}cm^{-1}$ | 75.37 | -0.241 |

*M.Wt.- Molecular weight; pI- Theoretical pI; EC- Extinction coefficient; AI- Aliphatic index; GRAVY- Grand Average of Hydropathicity.*

**Table.4 Physicochemical properties of phytase microbial sequences computed using Protparam tool**

| S. NO. | DESCRIPTION | ACCESSION NUMBER | NUMBER OF AMINO ACIDS | M.WT | pI | EC | AI | GRAVY |
|---|---|---|---|---|---|---|---|---|
| 1. | *Bacillus subtilis* | WP_019258689.1 | 382 | 41746.1 | 4.91 | 43320$M^{-1}cm^{-1}$ | 73.53 | -0.568 |
| 2. | *B.mojavensis* | WP_024121774.1 | 382 | 41798.2 | 5.10 | 46300$M^{-1}cm^{-1}$ | 76.88 | -0.579 |
| 3. | *B.amyloliquefaciens* | WP_045511202.1 | 383 | 41858.5 | 5.21 | 46300 $M^{-1}cm^{-1}$ | 70.89 | -0.593 |
| 4. | *B.methylotrophicus* | WP_046702299.1 | 383 | 41762.2 | 5.02 | 46300 $M^{-1}cm^{-1}$ | 69.87 | -0.603 |
| 5. | *B.siamensis* | WP_045926145.1 | 383 | 41878.5 | 5.31 | 46300 $M^{-1}cm^{-1}$ | 70.37 | -0.620 |

*M.Wt.- Molecular weight; pI- Theoretical pI; EC- Extinction coefficient; AI- Aliphatic index; GRAVY- Grand Average of Hydropathicity.*

Physico-chemical properties were examined to find differences between various sequences of phytase from plants and microbes using ExPASy'sProtParam tool.

The isoelectric point is the pH at which the protein does not migrate in an electric field. In our study, the pI value of the phytase from *Vigna radiata* was 5.11 and from *Bacillus subtilis* was 4.91 which indicated that it was acidic in nature. Similar studies were done in Dicer proteins. The computed pI value that was less than 7 (pI<7) indicates that proteins were considered as acidic and greater than 7 (pI>7) reveals that these dicer proteins were basic in character. The pI value of XP_003535104 and ABS32306 that are greater than 7 (pI>7) reveals that these proteins were basic in character [2].

The extinction coefficient (EC) indicates an amount of light absorption of proteins at a certain wavelength. Extinction coefficient of phytase from *Vigna radiata* was 77935 M–1cm–1 and from *Bacillus subtilis* was 43320 M-1 cm-1. The medium extinction coefficient indicates the presence of Cys, Trp and Tyr. These amino acids (Trp, Tyr, Cys) are considered to be an important parameter in the calculation of extinction coefficient of proteins [3].

The aliphatic index of a protein is a measure of the relative volume occupied by aliphatic side chain of the following amino acids: Alanine, valine, leucine and isoleucine. The aliphatic index of phytase from *Vigna radiata* was 68.61 and from *Bacillus subtilis* was 73.53 indicated that the protein may be stable for a wide range of temperatures.

The GRAVY (Grand Average of Hydropathy) value for protein is calculated as the sum of hydropathy values of all the amino acids. A hydropathy scale which is based on the hydrophobic and hydrophilic properties of the 20 amino acids is used. GRAVY values of phytase from *Vigna radiata* and *Bacillus subtilis* were -0.313 and -0.568. The very low GRAVY index indicated that it could have better interaction with water. The very low GRAVY index of DCLs EEE81952, XP_003553805 and XP_002268369 implies that these DCLs could result in a better interaction with water [2].

**Identification of domains**

By using CDD, identification of domains were done for the phytase from *Vigna radiata* and *Bacillus subtilis*. It was found that six domains were present in *Vigna radiata* and two domains present in *Bacillus subtilis*(CDD result page-Fig.1 and Fig.2).

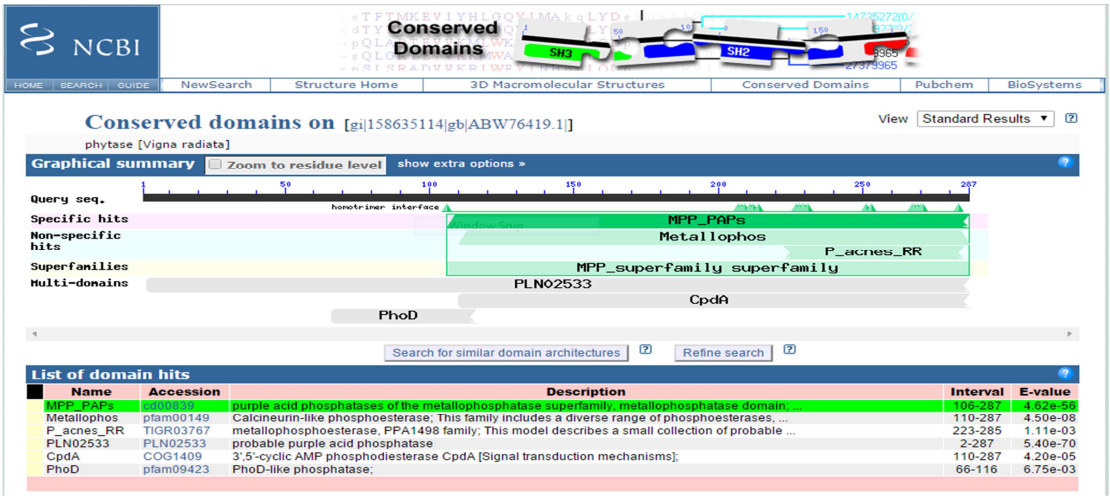


**Fig.1 Identification of domains from plants**

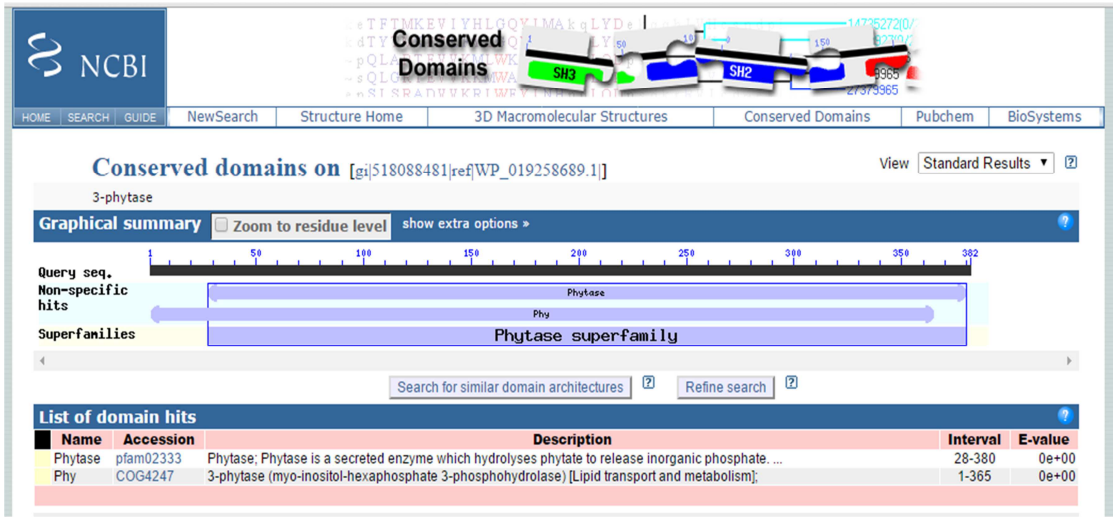


**Fig.2 Identification of domains from microbes**

The results coincide with the domain search done by Conserved domain search on the Blast site and it showed two domains – the medium chain reductase/dehydrogenases (MDR) / zinc dependent alcohol dehydrogenase like family and L-idonate 5 dehydrogenase family [9].

**Multiple sequence alignment (MSA) and Phylogenetic analysis**

Phylogenetic tree was constructed by NJ- Neighbour Joining method which resulted in a cladogram (Fig.3 and Fig.4).

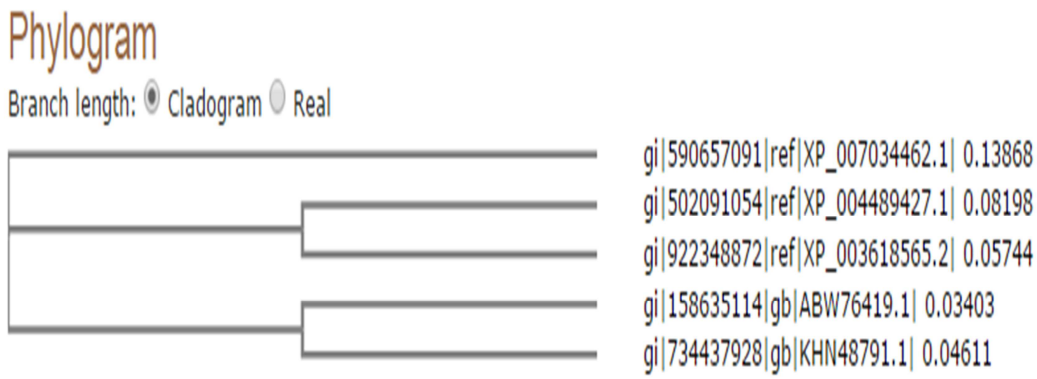


**Fig.3Cladogram of phytase of five plant sequences**

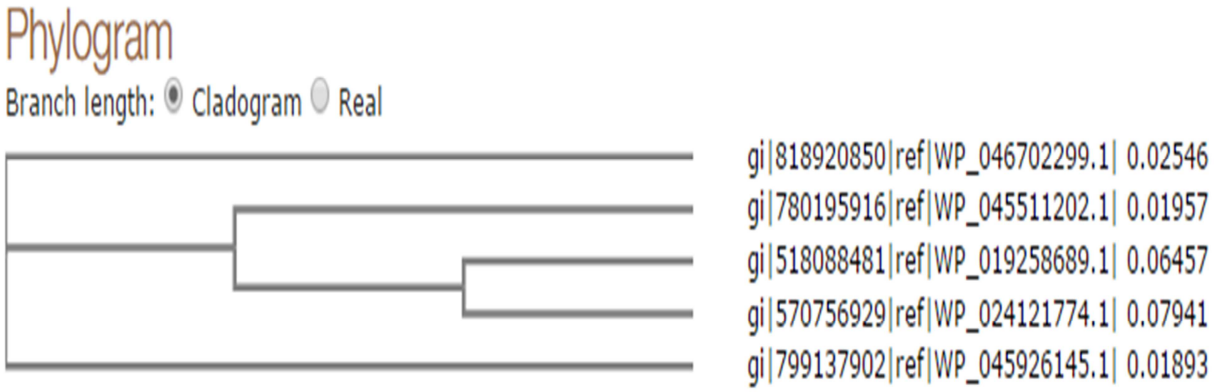


**Fig.4Cladogram of phytase of five microbial sequences**

Multiple sequence alignment (MSA) was done using Clustal Omega. It showed the relationship between the sequences.

The cladogram helps us to infer the evolutionary history of the selected sequence. A cladogram is a branching tree-like diagram in which the end points of the branches represent specific species of organisms.

The cladogram constructed showed that the sequence of phytase from *Vigna radiata*(ABW76419.1) and *Glycine soja*(KHN48791.1)are closely related among the five sets of plant sequences and *Bacillus subtilis*(WP_019258689.1) and *Bacillus mojavensis*(WP_024121774.1) are closely related among the five sets of microbial sequences.

## CONCLUSION

The present study clearly shows the various physico-chemical properties, biological parameters like domains of the phytase produced from these plants and microbes which are responsible for the functional hydrolysis of phytic acid. The study also revealed that the phytase production is found variable among the different species of the same genus (microbes) and also variation exists between the genus(plants). Thus the phytase produced from all these plant products and microbes can be recommended to food industry for the production of phytases in large scale and can be used in reducing the levels of phytic acid. Further, the enzyme can be recommended to be used as a nutrient supplement.

**Acknowledgement**
The authors thank Mrs. Prema Sampathkumar, Associate Professor and Head, the Faculty members and non-teaching staff of Department of Plant Biology and Plant Biotechnology, Ethiraj College for Women (Autonomous), Chennai- 600 008 and Dr. Mrs. A. Nirmala, Principal, Ethiraj College for women (Autonomous) for their valuable support, encouragement throughout the entire period of research.

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