DEVELOPMENT OF PHYTASEDB-AN ONLINE REPOSITORY OF SEQUENCE, STRUCTURE AND LITERATURE DATA RELATED TO PHYTASE

A PROJECT REPORT

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VANNAKUMAR .S (0810204051)

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COIMBATORE-641 047

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ANNA UNIVERSITY OF TECHNOLOGY, COIMBATORE
COIMBATORE – 641047

BONAFIDE CERTIFICATE

Certified that this project report **" DEVELOPMENT OF PHYTASEDB-AN ONLINE REPOSITORY OF SEQUENCE, STRUCTURE AND LITERATURE DATA RELATED TO PHYTASE"** is the bonafide work of "**VANNAKUMAR .S (Reg. No. 0810204051)"** who carried out the project work under my supervision.

SIGNATURE	**SIGNATURE**
SUPERVISOR	**HEAD OF THE DEPARTMENT**
Dr.VINOHAR STEPHEN RAPHEAL	**Dr. A. MANICKAM**
Associate Professor	Professor and Head
Department of Biotechnology	Department of Biotechnology
Kumaraguru College of Technology	Kumaraguru College of Technology
P. O. Box No. 2034	P. O. Box No. 2034
Chinnavedampatti	Chinnavedampatti
Coimbatore – 641 049	Coimbatore – 641 049
_____	_____
Internal Examiner	External Examiner

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(VANNAKUMAR .S)

ABSTRACT

Phytases catalyzes the hydrolysis of phosphorous of phytic acid. Seeds and grains are rich source of this enzyme. The released inorganic phosphate is utilized by the germinating seeds for biosynthesis of molecules like phospholipids and nucleic acids. Phytic acid is also an antinutrient in the dietary cereals that are consumed by humans and animals. It chelates minerals like calcium, iron and proteins. Industrially phytase therefore used to degrade phytic acid to increase bioavailability of nutrients. Commercial phytase is added as a supplement to swine and poultry feed to increase phosphorous and mineral bioavailability. Plant phytase has been used for food processing applications. Numerous groups have been actively carrying our studies on various aspects of phytase and a large body of information is publically available. The present study attempts to collect the scattered data from various databases, organize them and make the data accessible from one localized server via the internet. This database has been implemented in PHP and MySQL. The database will help disseminate phytase related sequence, structure and literature data in an accessible and efficient manner.

Availability: PhytaseDB is available for free at http://www.phytase.net63.net

Key words: phytase, structure, sequence, literature, PHP, MySQL.

TABLE OF CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
	List of Figures	viii
	List of abbreviations	x
1	**Introduction**	1
	1.1 General	1
	1.1.1 Phytase	1
	1.1.2 Biological Database	3
	1.2 Objectives	4
2	**Review of Literature**	5
	2.1 Phytase	5
	2.1.1 Microbial Sources	5
	2.1.2 Plant Sources	5
	2.1.3 Animal Sources	6
	2.2 Applications of Phytase	6
	2.2.1 Feed Application	6
	2.2.2Food Application	7
	2.2.3Preparation of *Myo*-Inositol Phosphates	8
	2.2.4Pulp and Paper Industry	9
	2.3 Biological Database	9
	2.3.1 Data collection & curation	9
	2.3.2 Design And Implementation	9
	2.4 Reference Database – Biodenz	10
	2.4.1 Database structure	10
	2.4.2 Development & website structure	10
	2.5 Reference Database-Peroxibase	11
	2.5.1 Framework	11
	2.5.2 Data Acquisition and Integration	12
	2.6 Reference Database - Phosphabase	13
	2.6.1 Database Content	13
	2.6.2 Data Extraction	13
	2.7 Reference Database - Histome	13
	2.7.1 Database and website implementation	14
	2.8 Reference Database - Thyme	15
	2.8.1 Content	15
	2.8.2 Database Organization and Future	15
	2.8 Data Sources	16
	2.8.1 GenBank	16
	2.8.2 EMBL	17
	2.8.3 DDBJ	17
	2.8.4 PubMed	17
	2.9 Protein Information Resource (PIR)	18
	2.9.1 UniProt	18
	2.9.2 iProClass	18
	2.9.3 PRO	19
	2.9.4 iProLINK	19
	2.10 JMOL	19
3	**Materials and Methods**	20
	3.1 Materials	20
	3.1.1 XAMPP	20
	3.1.2 Downloading and Installing XAMPP	21
	3.1.3 PHP	23
	3.1.3 National Center for Biotechnology Information	23
	3.1.3 Protein Data Bank (PDB)	24
	3.2 Methods	25
	3.2.1 Creating a Database	25
4	**Results and Discussion**	28
	4.1 Phytase Database	28
	4.2 Creating Dynamic Web pages	29
	4.3 Home page of Phytasedb	31
	4.4 PhytaseDB “Search by source” page	32
	4.5 PhytaseDB “Structure search and result” pages	33
	4.6 3D molecular view of the protein structure	34
	4.7 PhytaseDB “Sequence search and result” pages	35
	4.8 PhytaseDB “Abstracts search and result” pages	37
5	**Conclusion**	40
	Appendices	41
	References	59

LIST OF FIGURES

FIGURE NO	TITLE OF FIGURES	PAGE NO
1.1	The formation of myo-inositol phosphates from Phytate (Phytase)	2
2.1	Flow Sheet of BiodEnz Development	10
2.2	Phylogenic relationships between the protein classes in PeroxiBase	12
2.3	Relationship between tables in histone database	14
3.1	XAMPP Control Panel	22
3.2	XAMPP Control Panel Initialization	22
3.3	XAMPP Splash Screen	23
3.4	Screenshot of NCBI homepage	24
3.5	Screenshot of PDB homepage	24
3.6	PHPMyAdmin Start Page	26
3.7	Table creation Page	27
4.1	**Database Architecture**	28
4.2	**Data Flow Diagram**	29
4.3	Screenshot of phpMyAdmin database(Phytase)	30
4.4	Screenshot of PhytaseDB Home page	31
4.5	Screenshot of PhytaseDB **Search by Source using AJAX**	32
4.6	Screenshots of PhytaseDB **Structure search [A) Structure search by PDBID input page B)Output page C) Structure search by Keyword input page D)Output page]**	33
4.7	Screenshot of JMOL view showing the Ball and Stick 3D model of the selected Protein Structure	34
4.8	Screenshots of PhytaseDB **Sequence search [A) Sequence search by GenbankID input page B)Output page 1 showing list C) Output page 2 showing FASTA format of the Sequence]**	35
4.9	Screenshots of PhytaseDB **Sequence search [A) Sequence search**	36

by Keyword input page B)Output page showing paged results]

4.10 Screenshots of PhytaseDB **Abstract search [A) Abstract search by PubmedID input page B)Output page showing results C) Output page showing full Abstract]** 37

4.11 Screenshots of PhytaseDB **Abstract search [A) Abstract search output page showing Medline format B) Output page showing medline format download window]** 38

4.12 Screenshots of PhytaseDB **Abstract search [A) Abstract search by keyword input page B)Output page showing results]** 39

LIST OF ABBREVATIONS

DB	Database
DDBJ	DNA Data Bank of Japan
EMBL	European Molecular Biology Laboratory
ER	Entity Relationship
EC	Enzyme Commission
FTP	File Transfer Protocol
GUI	Graphical User Interface
HTML	Hyper Text Markup Language
HTTP	Hyper Text Transfer Protocol
SQL	Structure Query Language
NCBI	National Center for Biotechnology Information
NLM	National Library of Medicine
OMIM	Online Mendelian Interface in Man
PDB	Protein Data Bank
PERL	Practical Extraction and Report Language
PHP	HyperText PreProcesser
tRNA	Transfer Ribo Nucleic Acid
XAMPP	X (any of four different operating systems),Apache, MySQL, PHP, PERL
PRO	Protein Representing Objects
PIR	Protein Information Resource

CHAPTER 1
INTRODUCTION

1.1 GENERAL:

1.1.1 Phytase:

Phytases[myoinositol (1, 2, 3, 4, 5, 6) hexakisphosphatephosphohydrolases] have been identified in plants, microorganisms, and in some animal tissues. They represent a subgroup of phosphatases which are capable of initiating the stepwise dephosphorylation of phytate [myo-inositol (1, 2, 3, 4, 5, 6) hexakisphosphate], the most abundant inositol phosphate in nature. This classification is irrespective of their in vivo function, which remains usually unknown. Based on the catalytic mechanism, phytases can be referred to as histidine acid phytases, b-propeller phytases, cysteine phytases or purple acid phytases (2, 3). Depending on their pH optima, phytases have been divided into acid and alkaline phytases and based on the carbon in the myo-inositol ring of phytate at which dephosphorylation is initiated into 3-phytases (E.C. 3.1.3.8), 6-phytases (E.C. 3.1.3.26) and 5-phytases (E.C. 3.1.3.72).

The ruminants digest phytic acid through the action of phytases produced by the anaerobic gut fungi and bacteria present in their rumenal microflora. However, monogastric animals such as pig, poultry and fish utilize phytate phosphorus poorly because they are deficient in gastrointestinal tract phytases. Therefore, supplemental inorganic phosphate is added to thei feed to meet the phosphate requirement and to ensure good growth. However, supplemental inorganic phosphate does not diminish the antinutritive effect of phytic acid. The antinutritive effect of phytic acid is especially problematic in the feeding of fish (Richardson *et al*., 1985), due to their short gastrointestinal tract. This hinders the use of plant-derived protein in fish feed.

The problems mentioned above could be solved by hydrolysis of phytate using supplemental phytase. Therefore, phytase has become an important industrial enzyme and is the object of extensive research. By working efficiently on the substrate in the prevailing conditions, supplemental phytase could diminish the anti nutritive effects of phytic acid and reduce the cost of diets by removing or reducing the need for supplemental inorganic phosphate. In addition, phytase would be an environmentally friendly product, reducing the amount of phosphorus entering the environment. The Netherlands, Germany, Korea and Taiwan have enacted or are enacting legislation to reduce the phosphorus pollution created by monogastric livestock production (Wodzinski and Ullah, 1996).

Myo-inositol phosphates are also found in animal cells. However, the primary function of these compounds in animal cells is not to serve as a storage form of phosphorus or myo- inositol. Instead, their major role is in transmembrane signalling and mobilization of calcium from intracellular reserves. Therefore, these myo-inositol phosphates can be used as enzyme substrates for metabolic investigation, as enzyme inhibitors and therefore potentially as drugs (Laumen and Ghisalba, 1994). Chemical synthesis of these compounds is difficult, requiring protection and deprotection steps. Thus phytase, which converts phytic acid to lower myo-inositol phosphates, could be used for industrial production of these special myo-inositol phosphate derivatives.

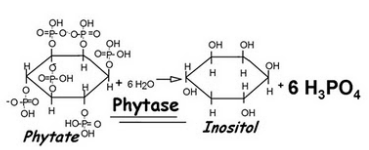


Fig 1.1 The formation of myo-inositol phosphates from Phytate (Phytase)

Phosphatases are a diverse class of enzymes catalyzing the cleavage of monophosphoester bonds in various organo-phosphate compounds. However, these enzymes are virtually unable to hydrolyze the monophosphoester bonds in phytic acid. Since the hydrolysis of phytic acid is of great importance a special class of enzymes hydrolyzing phytic acid has evolved the phytases.

1.1.2 Biological Databases:

When Sanger first discovered the method to sequence proteins, there was a lot of excitement in the field of Molecular Biology. Initial interest in Bioinformatics was propelled by the necessity to create databases of biological sequences. Biological databases can be broadly classified in to sequence and structure databases. Sequence databases are applicable to both nucleic acid sequences and protein sequences, whereas structure database is applicable to only Proteins. The first database was created within a short period after the Insulin protein sequence was made available in 1956. Incidentally, Insulin is the first protein to be sequenced. The sequence of Insulin consisted of just 51 residues (analogous to alphabets in a sentence) which characterize the sequence.

Around mid nineteen sixties, the first nucleic acid sequence of Yeast tRNA with 77 bases (individual units of nucleic acids) was found out. During this period, three dimensional structures of proteins were studied and the well known Protein Data Bank was developed as the first protein structure database with only 10 entries in 1972. This has now grown in to a large database with over 10,000 entries. While the initial databases of protein sequences were maintained at the individual laboratories, the development of a consolidated formal database known as SWISS-PROT protein sequence database was initiated in 1986 which now has about 70,000 protein sequences from more than 5000 model organisms, a small fraction of all known organisms. These huge varieties of divergent data resources are now available for study and research by both academic institutions and industries. These are made available as public domain information in the larger interest of research community through Internet. These databases are constantly updated with additional entries.

1.2 OBJECTIVES:

Objective of the present study are:

- ✓ To collect structural, sequence and literature data for phytase.
- ✓ To create ER model for the database and to implement it by using PHP and MySQL.
- ✓ To test the database in the local server using xampp and to publish it in the World Wide Web.

CHAPTER 2

2. REVIEW OF LITERATURE

2.1 PHYTASE:

2.1.1 Microbial Sources:

Microbial phytase activity is most frequently detected in fungi, particularly in *Aspergillus* species. (Shieh and Ware 1968) screened over 2000 microorganisms isolated from soil for phytase production. Most of the positive isolates produced only intracellular phytase. Extracellular phytase activity was observed only in 30 isolates. All extracellular phytase producers were filamentous fungi. Twenty-eight belonged to the genus *Aspergillus*, one to *Penicillum* and one to *Muco*, Of the 28 phytase-producing *Aspergillus* isolates 21 belonged to the *A. niger* group. Other studies (Howson and Davis, 1983; Volfova *et al*., 1994) confirmed A. *niger* strains to be the best producers of extracellular phytase.

Phytase has also been detected in various bacteria, e.g. *Aerobacter aerogenes* (Greaves *et al*., 1967), *Pseudomonas* sp. (Irving and Cosgrove, 1971), *Bacillus subtilis* (Powar and Jagannathan, 1982), *Klebsiella* sp, (Shah and Parekh, 1990), *B. subtilis* (natto) (Shimizu, 1992), *Escherichia coli*(Greiner *et al*., 1993) , *Enterobacter* sp(Yoon *et al*., 1996) and *Bacillus* sp. DS 11(Kim *et al*. 1998a). The only bacteria producing extracellular phytase are those of the genera *Bacillus* and *Enterobacter*. *E. coli* phytase is a periplasmic enzyme.

Some yeast, such as *Saccharomyces cerevisiae*, *Candida tropicalis*, *Torulopsis candida*, *Debaryomyces castelii*, *Debaryomyces occidentalis*, *Kluyveromyces fragilis* and *Schwanniomyces castelii*, have also been shown to produce phytase (Nayini and Markakis, 1984; Lambrechts *et al*., 1992).

2.1.2 Plant Sources:

Phytases occurs widely in the plant kingdom. Phytases has been isolated and characterized from cereals such as triticale, wheat, maize, barley and rice and from beans such as navy beans, mung beans, dwarf beans and California small white beans. Phytase activity has also been detected in white mustard, potato, radish, lettuce, spinach, grass and lily pollen (Dvorakova,J. 1998). Laboure *et al*, 1993) purified and characterized phytase from germinating maize seedlings (Zea mays), and the cDNA coding for this phytase was cloned (Maugenest *et al*., 1997).This cDNA was used to screen a maize genomic library and two distinct genes were isolated and sequenced.

2.1.3 Animal Sources:

Phytase has been found to exist in monogastric animals (Bitar and Reinhold, 1972; Copper and Gowing, 1983; Yang *et al*., 1991a; Chi *et al*., 1999).Generally; however, intestinal phytase does not play a significant role in food-derived phytate digestion in monogastrics (Williams and Taylor, 1985, Craxton *et al*. 1997) cloned and expressed a rat hepatic multiple inositol polyphosphate phosphatase (MIPP) having phytase activity. The MIPP mRNA was present in all rat tissues examined, but was most highly expressed in kidney and liver. A phytase-like enzyme was alsodecribed in the protozoan *Paramecium* (Freund *et al*., 1992).

2.2 APPLICATIONS OF PHYTASE:

2.2.1 Feed Application:

Ruminants digest phytate through the action of phytases produced by microbial flora in the rumen. The anaerobic gut fungi and bacteria present in the microflora of ruminants are responsible for the primary colonization of plant material within the rumen. The inorganic phoshate hydrolyzed from phytate by phytases is utilized by both the microflora and the ruminant host. The situation is different with monogastric animals. Monogastrics, such as pig, poultry and fish are unable to metabolize phytic acid, since they lack gastrointestinal phytase. Therefore, inorganic phosphate is added to their feed to meet the phosphate requirement. This increases costs and contibutes to phosphate pollution problems. The supplementation of animal feed with phytase enables the assimilation of phosphate in the feed ingredients and diminishes the amount of phosphate in the manure and subsequently reaching the environment. The effect of feeding phytase to animals on pollution has been quantitatively determined. If phytase were used in the feed of all of the monogastric animals reared in the U.S., it would release phosphorus with a value of 168 million U.S dollars and would preclude 8.23 $\times$ 10^4 tonnes of phosphate from entering the environment per annum. The use of phytase as a feed additive has been approved in 22 countries. The FDA (The Food and Drug Administration) has approved the phytase preparation as GRAS (Generally Regarded As Safe) (Wodzinski, and Ullah, 1996).Finase phytase added to corn-soybean pig diet converted approximately one-third of the

unavailable phosphate to an available form (Cromwell *et al*., 1993). In a similar way, experiments with Allzyme Phytase and Natuphos phytase additions to pig and chicken diet indicated that phytase improved the bioavailability of phytate phosphorus to pigs and broilers (Cromwell et *al*., 1993, b; Yi *et al*., 1996; O'Quinn *et al*., 1997). Several experiments with pigs and chickens confirmed the possibility to replace inorganic phosphate supplementation by the use of microbial phytase in phytate-rich diets for monogastric animals. In Holland, *A. niger* phytase has been successfully introduced as a feed supplement, leading to a 30 - 40% reduction in phosphate pollution (Jongbloed *et al*., 1992). This represents a nation-wide saving that is greater than the reduction obtained by phosphate-free detergent products (Quax, W.J 1997).

The use of phytase as a feed enzyme sets certain demands on the properties of the enzyme. Particularly, the enzyme should withstand high temperatures. This is because poultry and pig feed is commonly pelleted, which ensure that the animals have a balanced diet and facilitates the preservation of enzyme-containing product in the feed industry (Kim *et al*., 1999a). During the pelleting process the temperatures may temporarily reach 90 ^{o}C (Wyss *et al*., 1998).

2.2.2 Food Application:

A diet rich in cereal fibers, legumes and soy protein. Vegetarians, elderly people consuming unbalanced food with high amounts of cereals, people in undeveloped countries who eat unleavened bread and babies eating soy-based infant formulas take in large amounts of phytate. Undigested phytate in the small intestine negatively affects the absorption of zinc, calcium, magnesium and iron. It also reduces the digestability of dietary protein and inhibits digestive enzymes. Using Finase phytase, the preparation of a phytate-free soy protein isolate with increased solubility at low pH (pH 3) compared to the control soy protein isolate. (Anno *et al*.1985) eliminated phytate from soybean milk using wheat phytase. Additions of *A. niger* phytase to flour containing wheat bran increased iron absorption in humans (Sandberg *et al*., 1996).

2.2.3 Preparation of Myo-**Inositol Phosphates:**

The increasing interest in inositol phosphates and phospholipids, which play a pivotal role in transmembrane signalling and mobilization of calcium from intracellular reserves, has resulted in a need for various inositol phosphate preparations. Furthermore, specific inositol triphosphates have been suggested to prevent or alleviate diseases or conditions associated with abnormal levels of Neuropeptide Y (NPY). Among others, these include inflammatory diseases such as arthritis and respiratory diseases such as asthma. The use of specific inositol triphosphates as pain killers has also been proposed. Surprisingly, the esters of inositol triphosphate have been shown to exert significant inhibitory effects against retroviral infections including HIV.

The above-mentioned pharmaceutical applications of specific myo-inositol phosphates have further increased interest in the preparation of these compounds. The chemical syntheses of myo-inositol phosphates include difficult protection and deprotection steps, and are performed at extreme temperatures and pressures. Since phytases hydrolyze myo-inositol hexaphosphate sequentially, the production of myo-inositol phosphate derivates and free myo- inositol using phytase is a potential alternative to chemical synthesis. The preparation of D- myo-inositol 1, 2, 6-trisphosphate, D-myo-inositol 1, 2, 5-trisphosphate, L-myo-inositol 1,3,4- trisphosphate and myo-inositol 1,2,3-trisphosphate by enzymatic hydrolysis of phytic acid by *S. cerevisiae* phytase has been described . Immobilized phytases have been used to produce various myo-inositol phosphates (Ullah and Phillippy, 1988; Greiner and Konietzny, 1996). Naturally, the advantages of enzymatic hydrolysis are stereospecificity and mild reaction conditions. In addition to usage as drugs, myo-inositol phosphate derivatives can be used as enzyme substrates for biochemical and metabolic investigations and as chiral building blocks (Laumen and Ghisalba, 1994).

2.2.4 Pulp and Paper Industry:

It has been speculated that the removal of plant phytic acid might be important in the pulp and paper industry. A thermostable phytase could have potential as a novel biological agent to degrade phytic acid during pulp and paper processing. The enzymatic degradation of phytic acid would not produce carcinogenic and highly toxic by-products. Therefore, the exploitation of phytases in the pulp and paper process could be environmentally friendly and would assist in the development of cleaner technologies (Liu *etal*., 1998).

2.3BIOLOGICAL DATABASE:

2.3.1 Data Collection & Curation:

A literature search was done using PubMed and the journals like ScienceDirect, Springer link. From that all the available information is retrieved till date. Search terms included azo dye degrading enzymes Laccase, Lignin peroxidase and Azoreductase. The data has also been collected form the database Brenda (an enzyme database). Nucleotide sequences are collected from NCBI database and PDB ids are retrived from the Protein Data Bank. All the information has been curated manually (Ewan Birney and Michele Clamp, 2003)

2.3.2 Design and implementation:

Biological databases are essential to biological experimentation and analysis. They are used at different stages of life science research to deposit raw data, store interpretations of experiments and results of analysis processes, and search for matching structures and sequences. As such, they represent the backbone of life sciences discoveries. However, current database technology has not kept pace with the proliferation and specific requirements of biological databases. In fact, the limited ability of database engines to furnish the needed functionalities to manage and process biological data properly has become a serious impediment to scientific progress.

In many cases, biologists tend to store their data in flat files or spreadsheets mainly because current database systems lack several functionalities that are needed by biological databases, e.g., efficient support for sequences, annotations, and provenance. Once the data resides outside a database system, it loses effective and efficient manageability. Consequently, many of the advantages and functionalities that database systems offer are nullified and bypassed. It is thus important to break this inefficient and ineffective cycle by empowering database engines to operate directly on the data from within its natural habitat; the database system.

2.4 REFERENCE DATABASES – BIODENZ:

2.4.1 Database Structure:

The entries of our 'BiodEnz' database are generated from a text mining of hundreds of published articles.

2.4.2 Development & website structure:

The 'BiodEnz' database is developed using MySQL **[7**] a relational database management system that serves as the backend for storing data. APACHE 2.2 (Apache HTTP Server) is used as the web server and PHP5 (Hypertext preprocessor) a widely used scripting language driven by Zend engine is used as the web interface.

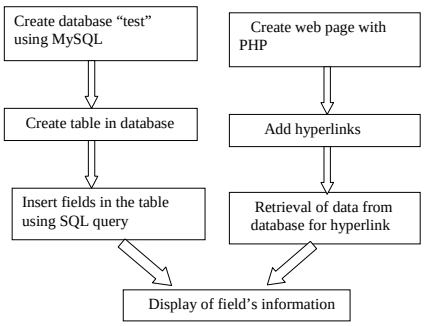


Fig 2.1 FlowSheet of BiodEnz Development

For the process of database creation in the PHP admin, the MySQL client version: 5.0.24a is used. The localhost of Server version: 5.0.24a, Protocol version: 10, Server: localhost via TCP/IP is used for the database creation. The local host for the process is phpMyadmin of 2.9.0 as shown in the **Figure1** (Shobana Sugumari and Berla Thangam, 2011).

2.5 REFERENCE DATABASE – PEROXIBASE:

2.5.1 Framework:

The phylogenetic trees of these 11 major groups can be subdivided in 60 subfamilies (Figure 2.2).These subdivisions based on evolution describe quite well the variety of peroxidase functions and can thus beused to predict the function of newly characterized proteins. Due to the high diversity of peroxidase functions and increased interest of the medical research in pathologies related to the role of peroxidases there is an urgent need to federate and organize data on peroxidases. The goal of our database is to centralize most sequences that belong to peroxidase super families, to follow the evolution of peroxidase among living organism and to compile the information concerning putative functions and transcriptional regulation. Currently, PeroxiBase is a unique repository exclusively dedicated to peroxidase families and super families from both Eukaryotes and Prokaryotes. It includes 6000 peroxidases encoding sequences from 940organisms, and each sequence is individually annotated.

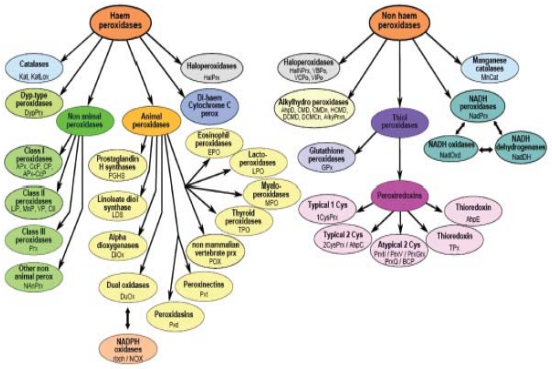


Fig 2.2

Phylogenic relationships between the protein classes in PeroxiBase.

2.5.2 Data Acquisition and Integration:

The automatic annotation of the complete genomes of numerous organisms and the automatic clustering and assembling of EST sequences led to the identification of numerous sequences coding for different peroxidase families and super families. However, the automatic processing of the sequences is known to be of poor quality or not as specific as expected. Using the highly conserved motifs of each peroxidase class, manual annotation and editing can clearly identify the correct sequences even in low-quality sequences. In order to increase data reliability, each new entry is individually controlled by a database curator. Each cross-reference is verified by the reviewer. The quality of the sequence is also examined by performing a sequence alignment with the other homologous sequences. (Dominique Koua *et al*., 2009)

modifying enzymes, hyperlinks were created to UniprotKB/Swiss-Prot, HGNC, OMIM, UniGene, RefSeq and other public databases. Internal hyperlinks were also created within the database pages wherever appropriate. These links greatly expand the annotation of Histome providing related knowledge from diverse sources.

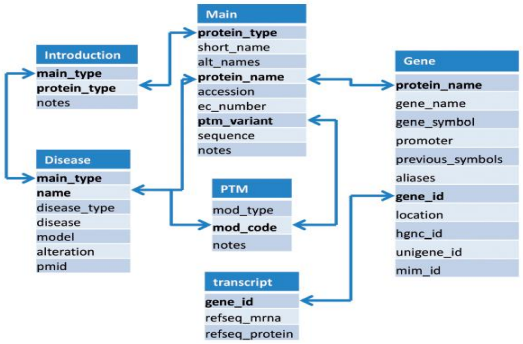


Fig 2.3 Relationship between tables in histone database

2.7.1 Database and website implementation:

Histome is available online and user friendly access is provided via a web interface. A detailed section has been added through side-menu on the contents of the database and how this resource can be used. A general introduction to chromatin and a detailed introduction to histones, their PTMs and modifying enzymes is available under respective menu elements. Information about different types of histones, their PTMs, various enzymes and likewise any specific entry can be retrieved in the database. The content of the database can be searched directly using any keyword(s) across the database using either a Google powered search or Histome advanced search (Satyajeet *et al.*, 2011).

2.8 REFERENCE DATABASE – THYME:

2.8.1 Content:

At present, ACSs are divided into five families, ATs intone, KSs into five, KRs into four, HDs into six, ERs into six and TEs into 23. ACCs are multi domain proteins first shown as organized into domains followed by each domain divided into families: one family of the biotin carboxylase (BC) domain, one family of the biotin carboxyl carrier protein (BCCP), and two families of the carboxyl transferase (CT) domain appear. These enzyme groups annotation and sequences in each family appear in ThYme organized in the way mentioned below.

2.8.2 Database organization and features:

The home page gives links to every enzyme group, as well as general information for viewers and citing and contact information. In each enzyme group's main page, all families are listed in a table with 'Names of enzymes and genes present', which presents a non-exhaustive overview of the sequences found. This is meant to guide new users to the family that contains their enzymes of interest. At the top of each enzyme family's page, it able gives general information about the family, describing protein folds (if known from crystal structures), the names of enzymes and genes present (the list is not exhaustive), EC numbers (the most common ones), the catalytic residues (if they are known from the literature), and other notes. Also shown is the total number of Protein Data Bank (PDB) structures, and enzymes with 'Evidence at protein level' and 'Evidence at transcript level' (see Experimentally Characterized sequences section below). This annotation might not be complete for all families. Within an enzyme family's page, all sequences appear by rows ordered into Achaea, bacteria and eukaryote, and alphabetically by producing species. All sequences in a row are identical and come from only one species. Identical sequences from different species are separated into different rows; however, identical sequences from different strains of the same species are not separated. If>500 rows exist, they are shown in multiple pages for a single family. The information is organized into the following columns: (i) names or designations given to the proteins; (ii) EC numbers assigned to them, with a link to the ExPASy proteomics server; (iii) genus and species names along with strain designations of the organisms that produced them, with a link to the National Center for Biotechnology Information (NCBI) taxonomy browser; (iv) their GenBank identification, with a link to the NCBI's protein database, their RefSeq identification, with a link also to the NCBI's protein database,their UniProt identification, with a link to the UniProt database and their PDB identification, with a link to the PDB, if their known tertiary structure is available. All sequence names and EC numbers are taken from either UniProt or NCBI's protein database; wedo not assign sequence names or EC numbers.Three features make navigating and retrieving information in ThYme easier. A search tool allows keywords, EC numbers

2.6 REFERENCE DATABASE –PHOSPHABASE:

2.6.1 Database Content:

The content and data structure of PhosphaBase was constructed in close collaboration with phosphatase biologists. Since the database will primarily be serving the phosphatase research community, the data contained within it had to reflect the needs of that community. As a result, the data in PhosphaBase range from the genomic level to three-dimensional (3D) protein structures. The current release has over 2800 phosphatase entries from345 different species.

All data in PhosphaBase have been populated with data extracted from peer-reviewed literature and from publicly

2.6.2 Data Extraction:

The data in PhosphaBase have been extracted from a number of different publicly available biological databases. Automatically extracting all relevant data without introducing any irrelevant data, and without omitting results, presented a number of problems. The same molecule can have multiple names, which can have multiple derivative abbreviations; for instance, protein-tyrosine phosphatase 1C has also been called protein-tyrosine phosphatase non receptor type 6, PTP-1C, hematopoietic cell protein-tyrosine phosphatase, 70Z-SHP, SH-PTP1, PTP-1C, and PTP1C. Key word searches that do not include all synonyms may result in the omission of data. Another problem common to most biological databases is that some of the information, often the most pertinent or important, is stored in free text, which is computationally unreadable. To overcome these problems in PhosphaBase, we do not rely on free-text and key word searches to extract data from biological resources. Instead, data are extract educing terms from the Gene Ontology. (K. J. Wolstencroft *et al*., 2005)

2.7 REFERENCE DATABASE – HISTOME:

Programs used to parse UniprotKB/Swiss-Prot XML files and output resulting data into MySQL tables were written in Python. Disease tables were generated manually by curating information obtained from literature. All data and information were stored in a MySQL relational database on a Linux server. Figure 1 shows a schematic layout of the database illustrating the links between different tables. Queries to the database were implemented in PHP scripts running in an Apache/PHP environment. The PHP scripting language enabled us to embed server-side code in XHTML documents. To annotate the functions of histone variants and their

and GenBank, RefSeq, UniProt or PDB accession codes to be searched. Furthermore, each family can be downloaded into a comma-separated value (csv) file, which can be viewed in a spreadsheet. Also, on each family's page, only rows that include a PDB link or a UniProt link marked with 'Evidence at transcript level'or 'Evidence at protein level' can be viewed. (David C. Cantu *et al*., 2010)

2.8 DATA SOURCES:

Biological data comes in many different flavors depending on the research project. Most researches work with a number of different formats even though they may not at first hand realize this. Often you have to look at sequence data, interpret gel analyses, look for related topics on PubMed and finally write everything together in a paper or a report. Some of these data in the databases are partly overlapping and referring to each other. Examples of text databases are PubMed and OMIM containing textual information and references related to biological Sequence data. GenBank and UniProt exemplify biological databases containing DNA and protein sequences, respectively. You can also find databases specifically related to protein structure files as e.g. the PDB, SCOP and CATH databases.

2.8.1 GenBank:

The GenBank sequence database continues to expandits data coverage, quality control, annotation contentand retrieval services. GenBank is comprised of DNAsequences submitted directly by authors as well assequences from the other major public databases. Anintegrated retrieval system,known as Entrez, containsdata from GenBank and from the major proteinsequence and structural databases, as well as related MEDLINE abstracts. Users may access GenBank overthe Internet through the World Wide Web and throughspecial client-server programs for text and sequencesimilarity searching. FTP, CD-ROM and e-mail servers are alternate means of access (Dennis *et al*., 1996)

2.8.2 EMBL:

The European Molecular Biology Laboratory (Embl) Nucleotide Sequence Database (http://www.ebi.ac.uk/embi/index.html) is maintained at the European Bioinformatics Institute (EBI) in an international collaboration with the Dna Data Bank of Japan (DDBJ) and Genbank(USA).Data is exchanged amongst the collaborative databases on adaily basis. The major contributors to the EMBL databases are individual authors and genome project groups. WEBIN is the preferred web-based submission system for individual submitters. Whilst automatic procedures allow incorporation of sequence data from large-scale genome sequencing centres and from the European Patent Office(EPO).Datadase releases are produced quarterly.Network services allow free access to the most up-to-date data collection via internet and WWW interfaces.EBI's Sequence Retrieval System(SRS) is a network browser for databanks in molecular biology,integrating and linking the main nucleotide and protein databases plus many specialized databases .For sequence similarity searching a variety of tools (e.g., BLITZ,FASTA,BLAST) are available which allow external users to compare their own sequences against the most currently available data in the EMBL Nucleotide Sequence Database and SWISS-PORT(Wendy Baskar *et al*.,2000)

2.8.3 DDBJ:

DDBJ (DNA Data Bank of Japan) is a nucleotide database hosted in Japan and is accepting DNA submission from mainly Japanese researchers. They work in close collaboration with GenBank and EMBL and the three databases store almost identical data. DDBJ also provides various search and analysis tools through the website http://www.ddbj.nig.ac.jp/.

2.8.4 PubMed:

PubMed gives you biological data in text format and this service provided by the U.S. National Library of Medicine links to more than 17 million resources from different journals within the field of life science. A relatively new functionality at the NCBI website is the possibility to sign up for an account at My NCBI which is a service that offering a customized and automated PubMed update. After registration at My NCBI you can save your searches and set up automated searches alerting you by e-mail. You can also customize e.g. filtering options on the searches. PubMed can be accessed at http://www.ncbi.nlm.nih.gov/pubmed/.

2.9 PROTEIN INFORMATION RESOURCE (PIR):

Integrated Protein Informatics Resource for Genomic, Proteomic and Systems Biology Research for over four decades the Protein Information Resource (PIR) has provided databases and protein sequence analysis tools to the scientific community, including the Protein Sequence Database, which grew out from the Atlas of Protein Sequence and Structure, edited by Margaret Dayhoff [1965-1978]. Currently, PIR major activities include: i) UniProt (Universal Protein Resource) development, ii) iProClass protein data integration and ID mapping, iii) PRO protein ontology,and iv) iProLINK protein literature mining and ontology development.

2.9.1 UniProt:

UniProt is the central resource for storing and interconnecting information from large and disparate sources and the most comprehensive catalog of protein sequence and functional annotation. UniProt used to retrieve curated, reliable, comprehensive information on proteins. Use UniRef to decrease redundancy and speed up sequence similarity searches. Use UniParc to access to archived se-quences and their source databases.

2.9.2 iProClass:

iProClass provides extensive data inte-gration of over 90 biological databases, with protein ID mapping service, and ex-ecutive summary descriptions of pro-teins for UniProtKB and selected Uni-Parc protein sequences. iProClass used to retrieve comprehen-sive, upto-date information about a pro-tein, including function, pathway, inter-actions, family classification, structure and structural classification, gene and genomes, ontology, literature, and tax-onomy. Use iProClass to access to ID mapping, protein BioThesaurus and re-lated sequences.

2.9.3 PRO:

PRO is a formal representation of protein objects, providing both descriptions of these objects and the relationships between them. PRO encompasses sub-ontology of proteins based on evolutionary relatedness (ProEvo) and sub-ontology of the multiple protein forms produced from a given gene (ProForm). PRO is interoperable with other OBO Foundry ontologies--such as the Sequence Ontology (SO) and the Gene Ontology (GO)--that provide representations of protein qualities. This interoperability facilitates cross-species compari-sons, pathway analysis, disease modeling, and the generation of new hypotheses through data integra-tion and machine reasoning.

2.9.4 iProLINK:

iProLINK provides annotated literature, protein name dictionary, and other infor-mation to facilitate Natural Language Processing technology development in literature mining, database curation, protein name tagging and ontology. iProLINK used to obtain literature sources that describe protein entries (BibliographyMapping), to map protein/gene names to UniProtKB entries (BioThesaurus), to ob-tain annotated data sets for developing text mining algorithms, to mine literature for protein phosphorylation (RLIMS-P).

2.10 JMOL:

Molecular visualization is a traditional example of a domain-specific visualization application. Molecular visualization environments tightly integrate data generation, visualization, and analysis.

JMol is an open-source molecular visualization program.Chemical scientist's use Jmol as a visualization and measurement tool. It is capable of animating the results of simulations and can perform transformations (rotation,translation, scaling) upon a molecular structure. In addition,the application can also measure inter-atomic distances, bond angles, and dihedral angles, as well as print and export captured images(Egon Willighagen and Miguel Howard, 2005).

Jmol is an event-driven system with its graphical user interface (GUI) controlling most events. Jmol's GUI is based on Java's Swing interface toolkit (JDK 1.2 and later), a refined version of Java's Abstract Window Toolkit (AWT) found in Java's JDK 1.1 and later. Each interface component, or widget, is linked to a listener interface that captures a unique event. Thus, when the mouse is moved, or a button is pushed, or a dropdown menu item is selected, an event occurs that is captured and acted upon.

And Java's listener interface provides specific methods in which to embed actions to be taken once an event has been captured. By finding the Java methods in Jmol that control the listener interface it should be possible to insert the JXTA code necessary to transmit an event and its attributes to a peer. Jmol's interface events result in loading and saving files, updating the molecular graphic representation, and generating geometric data to describe inter-atomic distances and angles. After the first peer acts on the event by updating its local state, it should send a unique message to the second peer for it to replicate the process. For example, the sending peer captures combined button and mouse events for translating the molecular representation, and redraws it at a new screen location. After translation,a message is sent to the receiving peer, corresponding to a translation action. The receiving peer then updates its state to match the sending peer

CHAPTER 3
MATERIALS AND METHODS

3.1 MATERIALS:

3.1.1 XAMPP:

XAMPP stands for X (any of four different operating systems), Apache, MySQL (Structured Query Language), PHP (Personal Home Page) and PERL (Practical Extraction and Report Language). It's not easy to install an Apache web server and it gets harder if you want to add MySQL, PHP and PERL. XAMPP is an easy to install Apache distribution containing MySQL, PHP and PERL. XAMPP is really very easy to install and to use - just download, extract and start.

There are four XAMPP distributions:

- The distribution for Linux systems (tested for SuSE, RedHat, Mandrake and Debian) contains: Apache, MySQL, PHP & PEAR, PERL, ProFTPD, PHPMyAdmin, OpenSSL, GD, Freetype2, libjpeg, libpng, gdbm, zlib, expat, Sablotron, libxml, Ming, Webalizer, pdf class, ncurses, mod_PERL, FreeTDS, gettext, mcrypt, mhash, eAccelerator, SQLite and IMAP C-Client.

- The distribution for Windows systems contains: Apache, MySQL, PHP & PEAR, PERL, mod_PHP, mod_PERL, mod_ssl, OpenSSL, PHPMyAdmin, Webalizer, Mercury Mail Transport System for Win32 and NetWare Systems v3.32, Ming, JpGraph, FileZilla FTP Server, mcrypt, eAccelerator, SQLite, and WEB-DAV & mod_auth_mySQL.

- The distribution for Mac OS X contains: Apache, MySQL, PHP & PEAR, SQLite, PERL, ProFTPD, PHPMyAdmin, OpenSSL, GD, Freetype2, libjpeg, libpng, zlib, Ming, Webalizer, mod_PERL.

- The distribution for Solaris (developed and tested with Solaris 8, tested with Solaris 9) contains: Apache, MySQL, PHP & PEAR, PERL, ProFTPD, PHPMyAdmin, OpenSSL, Freetype2, libjpeg, libpng, zlib, expat, Ming, Webalizer, pdf class.

XAMPP also provides support for creating and manipulating databases in MySQL.

3.1.2 Downloading and Installing XAMPP:

All XAMPP packages and add-ons are distributed through the Apache Friends website at the address: http://www.apachefriends.org/. Once on the website, navigate and find the Windows version of XAMPP and download the self-extracting ZIP archive. After downloading the archive, run and extract its contents into the root path of a hard disk or USB drive. For example, the extract path for a local Windows installation would simply be C:\. If extracted properly a new XAMPP directory was noticed in the root of installation disk. In order to test that everything has been installed correctly, first start the Apache HTTP Server by navigating to the XAMPP directory and run the apache_start.bat batch file. Next step is to open the XAMPP Control panel(Fig 3.1) and make sure that Apache, MySql, FileZilla and Mercury are started(Fig 3.2).

Next it should be tested if the server is running correctly by opening an internet browser and typing http://localhost/ into the address bar. If configured correctly, then the following screen will appear.

In order to stop all Apache processes do not close the running terminal application, but instead run another batch file in the XAMPP directory called apache_stop.bat (Dalibor, D. Dvorski, 2007).

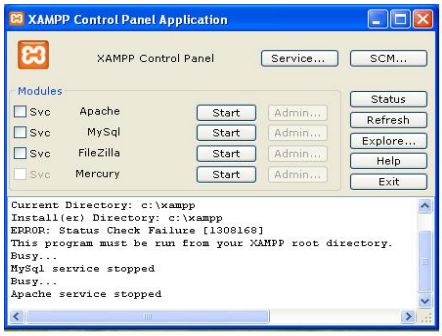


Fig 3.1 XAMPP Control Panel

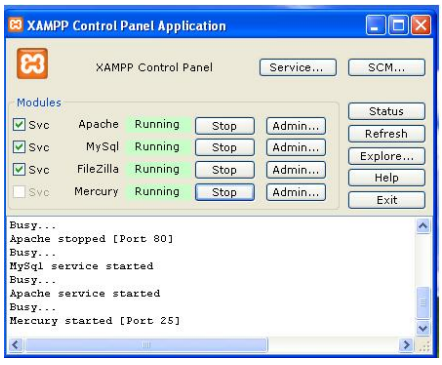


Fig 3.2 XAMPP Control Panel Initialization

Fig 3.3 XAMPP Splash Screen

3.1.3 PHP:

PHP is an open-source server-side scripting language. PHP is a widely-used general-purpose scripting language that is especially suited for Web development and can be embedded into HTML. The PHP scripting language resembles JavaScript, Java, and Perl. These languages all share a common ancestor, the C programming language.

3.1.4 National Center for BIotechnology Information (NCBI):

The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.

The National Center for Biotechnology Information (NCBI) is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health. The NCBI is located in Bethesda, Maryland and was founded in 1988 through legislation sponsored by Senator Claude Pepper. The NCBI houses genome sequencing data in GenBank and an index of biomedical research articles in Pub Med Central and Pub Med, as well as other information relevant to biotechnology. All these databases are available online through the Entrez search engine.

Fig 3.4 Screenshot of NCBI homepage

3.1.5 Protein Data Bank (PDB):

Fig 3.5 Screenshot of PDB homepage

The PDB archive contains information about experimentally-determined structures of proteins, nucleic acids, and complex assemblies. As a member of the PDB, the RCSB PDB curates and annotates PDB data according to agreed upon standards.

The RCSB PDB also provides a variety of tools and resources. Users can perform simple and advanced searches based on annotations relating to sequence, structure and function. These molecules are visualized, downloaded, and analyzed by users who range from students to specialized scientists.

The PDB is a key resource in areas of structural biology, such as structural genomics. Most major scientific journals, and some funding agencies, such as the NIH in the USA, now require scientists to submit their structure data to the PDB. If the contents of the PDB are thought of as primary data, then there are hundreds of derived (i.e., secondary) databases that categorize the data differently.

3.2 METHODS:

3.2.1 Creating a Database:

The XAMPP package contains an application called PHPMyAdmin which allows developers to administer and maintain MySQL databases. In order to start PHPMyAdmin, PHPMyAdmin folder is copied first from the XAMPP directory and is placed it in the XAMPP\htdocs directory. Before testing PHPMyAdmin, make sure that both Apache and MySQL are running by opening their respective batch files: apache_start.bat and mySQL_start.bat. Along with Apache and MySQL running in the background, type http://localhost/PHPMyAdmin/ into your internet browser. If successful PHPMyAdmin will be started with a start page similar to the one in Fig 3.6.

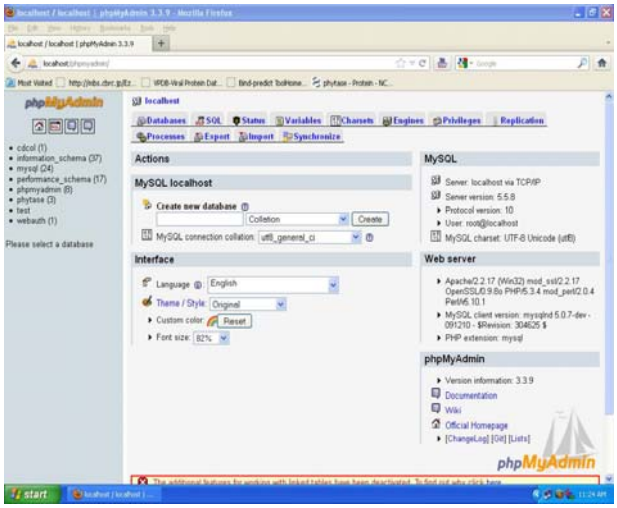


Fig 3.6 PHPMyAdmin Start Page

The first step with PHPMyAdmin is creation of a new database. New database is created by typing “Phytase” (Name of the database) in the textbox. New table is inserted by mentioning the name and number of fields in the table in the boxes provided. Press enter or clicks go to navigate to the next page. Attributes of the table and its contents is mentioned in the page as shown in Fig 3.7.

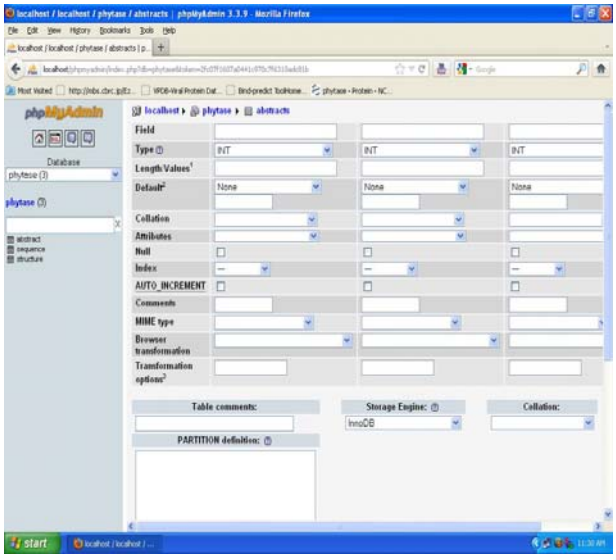


Fig 3.7 Table creation Page

The required details are typed in and thus the table with required fields is created. Similarly all the other required tables are created and the data’s are entered

CHAPTER 4
RESULTS AND DISCUSSION

4.1 PHYTASE DATABASE:

Phytase Database contains a total of 895 entries (89 sequence data's,31 structural data's and 775 literature/abstract data's) as shown in figure 4.1.Each table contains 3fields.Since pubmedID, PDBID and genbankID of abstracts, structure and sequence respectively cannot be null, CONSTRAINTS of NOT NULL and UNIQUE are given to these fields.Data entered in the table can be modified or deleted at any time.New data can be also be inserted into any of the fields of any table. Hence whenever a data about phytase is found, it can be updated and made available to the scientific community.

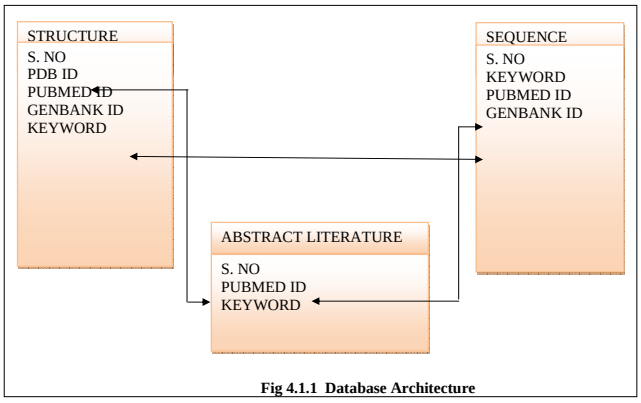


Fig 4.1.1 Database Architecture

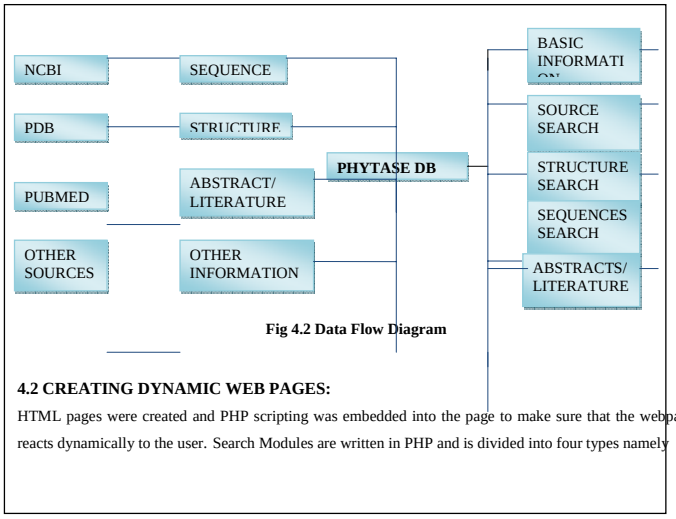


Fig 4.2 Data Flow Diagram

4.2 CREATING DYNAMIC WEB PAGES:

HTML pages were created and PHP scripting was embedded into the page to make sure that the webpage reacts dynamically to the user. Search Modules are written in PHP and is divided into four types namely

- ✓ Search by source,
- ✓ Structure search,
- ✓ Sequence search and
- ✓ Abstracts/Literature search.

The user can select any of the searches based on their requirements. Search by source is implemented using AJAX and the output will display the information of phytase from the source we select from the dropdown menu available. In structure search, user can either search by using PDBID or keyword. The output page contains all the structural information including primary citation and downloadable PDB file. It contains additional feature of 3D molecular visualization of the PDB file using JMOL. In sequence search, users can search by genbankID or keyword. The output page contains FASTA format of the selected sequence.

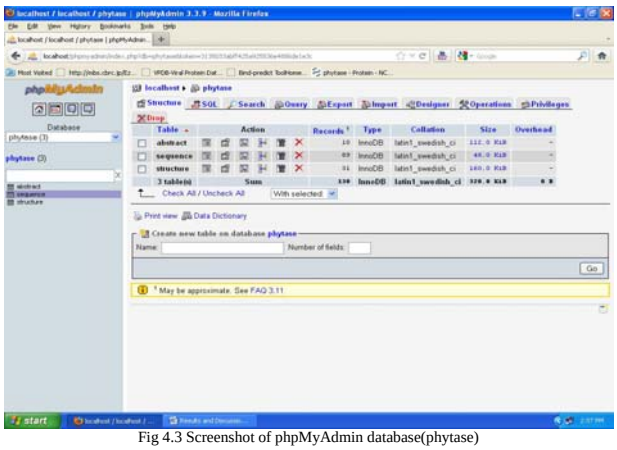


Fig 4.3 Screenshot of phpMyAdmin database(phytase)

4.3 HOME PAGE OF PHYTASEDB:

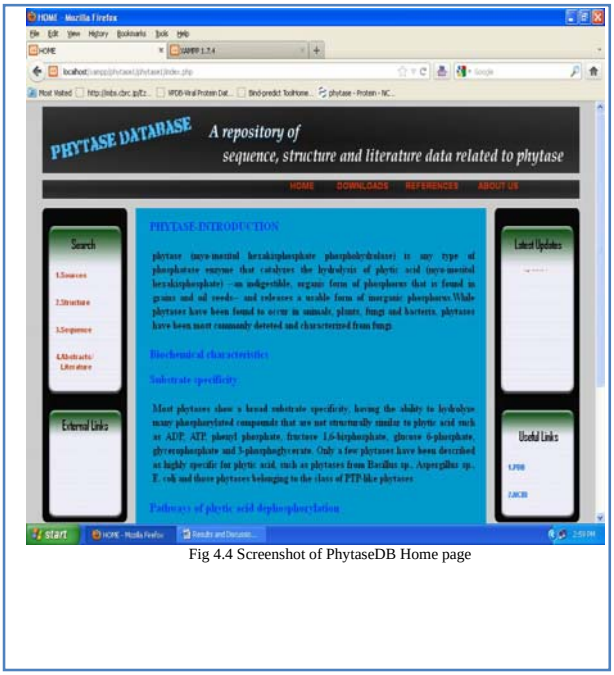


Fig 4.4 Screenshot of PhytaseDB Home page

4.4 PHYTASEDB "SEARCH BY SOURCE" PAGE:

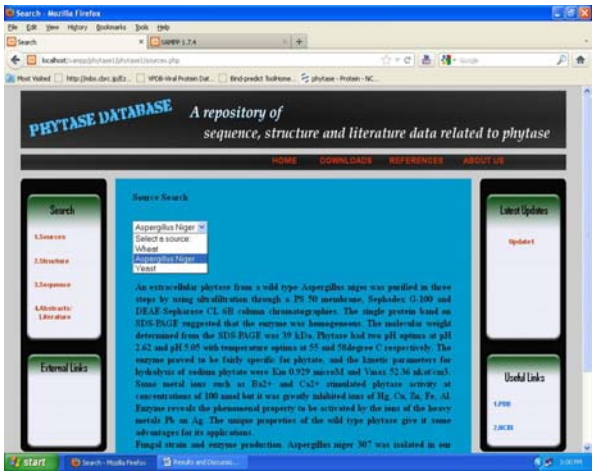


Fig 4.5 Screenshot of PhytaseDB Search by Source using AJAX

4.5 PHYTASEDB "STRUCTURE SEARCH AND RESULT" PAGES:

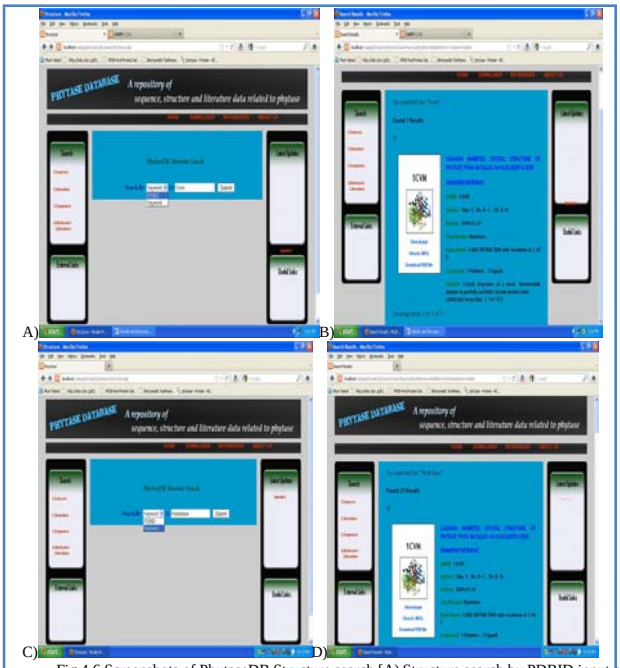


Fig 4.6 Screenshots of PhytaseDB Structure search [A) Structure search by PDBID input page B)Output page C) Structure search by Keyword input page D)Output page]

4.6 3D MOLECULAR VIEW OF THE PROTEIN STRUCTURE:

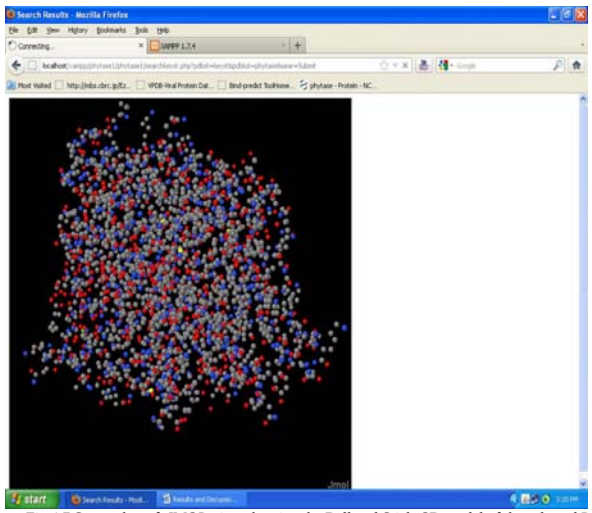


Fig 4.7 Screenshot of JMOL view showing the Ball and Stick 3D model of the selected Protein Structure

4.7 PHYTASEDB "SEQUENCE SEARCH AND RESULT" PAGES:

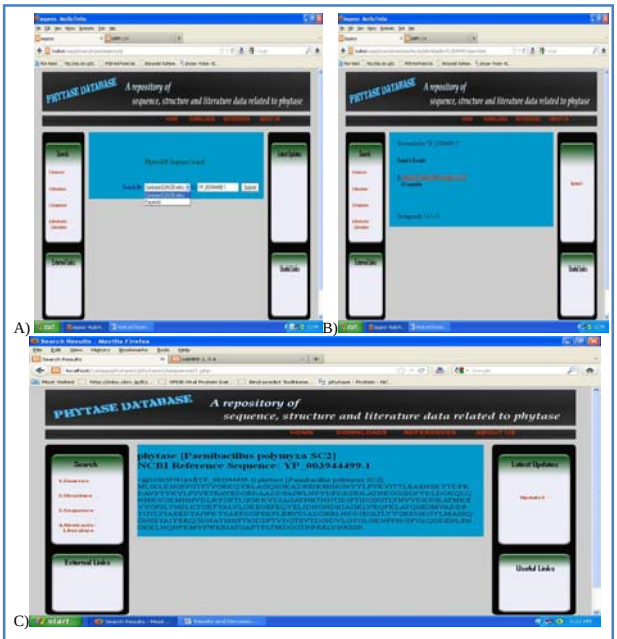


Fig 4.8 Screenshots of PhytaseDB Sequence search [A) Sequence search by GenbankID input page B) Output page 1 showing list C) Output page 2 showing FASTA format of the Sequence]

A)

B)

Fig 4.9 Screenshots of PhytaseDB Sequence search [A) Sequence search by Keyword input page B)Output page showing paged results]

4.8 PHYTASEDB "ABSTRACTS SEARCH AND RESULT" PAGES:

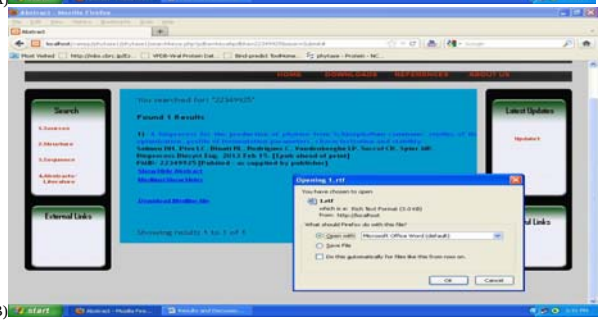
A)

B)

C)

Fig 4.10 Screenshots of PhytaseDB Abstract search [A) Abstract search by PubmedID input page B) Output page showing results C) Output page showing full Abstract]

A)



B)

Fig 4.11 Screenshots of PhytaseDB Abstract search [A) Abstract search output page showing Medline format B) Output page showing medline format download window]

A)

B)

Fig 4.12 Screenshots of PhytaseDB Abstract search [A) Abstract search by keyword input page B) Output page showing results]

CHAPTER 5

CONCLUSIONS AND FUTURE DEVELOPMENT

PhytaseDB's aim is to provide an easy access to phytases for researcher working in food industries and for other users of scientific community. The database will be periodically updated and enriched with more features to make it further interactive and user friendly. Tools like BLAST, CLUSTALW are to be incorporated in the database to equip the research community. Databases of related enzymes will also be created for better accessibility. The database is available for free at http://phytase.net63.net.

APPENDICES

APPENDIX 1:

A 1.1 Abstract table created using MySQL Queries in phpMyadmin:

sno	pubmedid	keyword
1	22349925	<b style="color:#0033FF"> A bioprocess for the pro...
2	22334743	<b style="color:#0033FF">In vitro evaluation of li...
3	22282635	<b style="color:#0033FF">Phytase, phosphatase acti...
4	22281295	<b style="color:#0033FF">Heterologous expression a...
5	22244916	<b style="color:#0033FF">Purification and characte...
6	22159661	<b style="color:#0033FF">Directed evolution of a h...
7	22147481	<b style="color:#0033FF">Digestibility of phosphor...
8	22112406	<b style="color:#0033FF">Site-directed mutagenesis...
9	22112273	<b style="color:#0033FF">Adsorption immobilization...
10	22101445	<b style="color:#0033FF">Improving Phytase Enzyme ...

A 1.2 Phytase Sequences table:

sno	genbankid	keyword
1	YP_003944499.1	<a class="key" href="sequence/1.php"> phytase [Pae...
2	ZP_09672975.1	<a class="key" href="sequence/1.php"> phytase [Myr...
3	ZP_09612508.1	<a class="key" href="sequence/1.php"> phytase [Muc...
4	YP_002014808.1	<a class="key" href="sequence/1.php"> phytase [Pro...
5	YP_001869795.1	<a class="key" href="sequence/1.php"> phytase [Nos...
6	XP_001827233.1	<a class="key" href="sequence/1.php"> phytase [Asp...
7	XP_001823745.1	<a class="key" href="sequence/1.php"> phytase [Asp...
8	YP_001362609.1	<a class="key" href="sequence/1.php"> phytase [Kin...
9	YP_869843.1	<a class="key" href="sequence/1.php"> phytase [She...
10	YP_696211.1	<a class="key" href="sequence/1.php"> phytase [Clo...
11	YP_633476.1	<a class="key" href="sequence/1.php"> phytase [Myx...
12	YP_201138.1	<a class="key" href="sequence/1.php"> phytase [Xan...
13	NP_925045.1	<a class="key" href="sequence/1.php"> phytase [Glo...
14	NP_718111.1	<a class="key" href="sequence/1.php"> phytase [She...
15	NP_389861.1	<a class="key" href="sequence/1.php"> phytase [Bac...
16	YP_001943170.1	<a class="key" href="sequence/1.php"> phytase [Chl...
17	ZP_06588929.1	<a class="key" href="sequence/1.php"> phytase [Str...

A 1.3 Phytase Structures table:

sno	pdbid	keyword
1	2GFI	<script type="text/javascript"> function jmolapp(...
2	2WNI	<script type="text/javascript"> function jmolapp(...
3	3K4P	<script type="text/javascript"> function jmolapp(...
4	1POO	<script type="text/javascript"> function jmolapp(...
5	2WNH	<script type="text/javascript"> function jmolapp(...
6	2WU0	<script type="text/javascript"> function jmolapp(...
7	1CVM	<script type="text/javascript"> function jmolapp(...
8	2POO	<script type="text/javascript"> function jmolapp(...
9	1QWO	<script type="text/javascript"> function jmolapp(...
10	3K4Q	<script type="text/javascript"> function jmolapp(...
11	1U24	<script type="text/javascript"> function jmolapp(...

A 1.3 Sample Structure of a Database table:

Field	Type	Collation	Attributes	Null	Default	Extra	Action
sno	int(5)			No	None	AUTO_INCREMENT	
pubmedid	varchar(1000)	latin1_swedish_ci		No	None		
keyword	mediumtext	latin1_swedish_ci		No	None		

APPENDIX 2:

A 2.1 Source code for HOME page:

```
<!DOCTYPE html PUBLIC "-//W3C//DTD XHTML 1.0 Transitional//EN"
"http://www.w3.org/TR/xhtml1/DTD/xhtml1-transitional.dtd">
<html xmlns="http://www.w3.org/1999/xhtml">
<head>
<meta http-equiv="Content-Type" content="text/html; charset=iso-8859-1" />
<title>HOME</title>
<link href="css/1.css" rel="stylesheet" type="text/css" />
</head><body>
<div id="cointainer">
<div id="img"><br/><br/><br/><br/><br/></div>
<div id="content">
<a href="index.php" style="margin-left:438px; font-size:14px; font-family:Arial, Helvetica,
sans-serif"><b>HOME</b></a><a href="downloads.php" style="margin-left:40px; font-
size:14px; font-family:Arial, Helvetica, sans-serif"><b>DOWNLOADS</b></a><a
href="references.php" style="margin-left:30px; font-size:14px; font-family:Arial, Helvetica,
sans-serif"><b>REFERENCES</b></a><a href="aboutus.php" style="margin-left:35px; font-
```

```
<tr><h6 style="font-family:arial"
style="font:color:#000066">        <a
href="structure.php">2.Structure</a></h6></tr>
<tr><h6 style="font-family:arial"
style="font:color:#000066">        <a
href="sequence.php">3.Sequence</a></h6></tr>
<tr><h6 style="font-family:arial"
style="font:color:#000066">        <a
href="abstracts.php">4.Abstracts/<br/>       &n
bsp;    Literature</a></h6></tr><tr></tr><tr></tr><tr> </tr>
<tr><h3 class="a2" style="font-family:'Agency FB'"
style="font:color:#000066">         
;External Links</h3></tr>
<tr><h3 class="a2" style="font-family:'Agency FB'"
style="font:color:#000066">    </h3></tr>
<tr><h3 class="a2" style="font-family:'Agency FB'"
style="font:color:#000066">    </h3></tr>
<tr><h3 class="a2" style="font-family:'Agency FB'"
style="font:color:#000066">    </h3></tr>
<tr><h3 class="a2" style="font-family:'Agency FB'"
style="font:color:#000066">    </h3></tr>
</table></div>
<div class="tab1 center"><h2 style="font-size:18px; color:#0033FF; margin-left:25px;
margin-top:15px;"><b>Phytase Introduction</b></h2><h4 style="margin-left:30px; margin-
right:20px;">   phytase (myo-inositol hexakisphosphate phosphohydrolase) is
any type of phosphatase enzyme that catalyzes the hydrolysis of phytic acid (myo-inositol
hexakisphosphate) --an indigestible, organic form of phosphorus that is found in grains and oil
seeds-- and releases a usable form of inorganic phosphorus.While phytases have been found to
occur in animals, plants, fungi and bacteria, phytases have been most commonly deteted and
characterized from fungi.</h4>
```

```
<div class="tab1">
<table><br/>
<tr><h3 class="a1" style="font-family:'Agency FB';text-decoration:blink;
">         Latest Updates</h3></tr>
<tr><h6 style="font-family:arial" style="font:color:#000066"><a
href="aboutus.php"><marquee direction="up" onmouseover="this.setAttribute('scrollamount',
0, 0);" onmouseout="this.setAttribute('scrollamount', 6,
0);">            
      Update1</marquee></a></h6></tr>
<tr><h3 class="a2" style="font-family:'Agency FB'"
style="font:color:#000066">         
;  Useful Links</h3></tr>
<tr><h6 style="font-family:arial"
style="font:color:#000066">        <a
href="http://www.rcsb.org" target="_blank">1.PDB</a></h6></tr>
<tr><h6 style="font-family:arial"
style="font:color:#000066">        <a
href="http://www.ncbi.nlm.nih.gov" target="_blank">2.NCBI</a></h6></tr><br/>
<tr>      </tr><br/>
<tr>      </tr><br/>
<tr>      </tr><br/>
<tr>      </tr><br/>
<tr>      </tr><br/>
<tr>      </tr><br/>
</table></div>
</div>
</body>
</html>
```

```
<h2 style="font-size:18px; color:#0033FF; margin-left:25px; margin-top:15px;">
Biochemical characteristics</br>Substrate specificity</h2>

<h4 style="margin-left:30px; margin-right:20px;">A mutation in the tyrosinase gene resulting
in impaired tyrosinase production leads to type I oculocutaneous albinism, a hereditary
disorder that affects one in every 17,000 people.</h4>
<h4 style="margin-left:30px;  margin-right:20px;">
Most phytases show a broad substrate specificity, having the ability to hydrolyze many
phosphorylated compounds that are not structurally similar to phytic acid such as ADP, ATP,
phenyl phosphate, fructose 1,6-bisphosphate, glucose 6-phosphate, glycerophosphate and 3-
phosphoglycerate. Only a few phytases have been described as highly specific for phytic acid,
such as phytases from Bacillus sp., Aspergillus sp., E. coli and those phytases belonging to the
class of PTP-like phytases</h4>
<h2 style="font-size:18px; color:#0033FF; margin-left:25px; margin-
top:15px;"><b>Pathways of phytic acid dephosphorylation</b></h2>
<h4 style="margin-left:30px; margin-right:20px;">
Phytic acid has six phosphate groups that may be released by phytases at different rates and in
different order. Phytases hydrolyze phosphates from phytic acid in a stepwise manner,
yielding products that again become substrates for further hydrolysis. Most phytases are able
to cleave five of the six phosphate groups from phytic acid. Phytases have been grouped based
on the first phosphate position of phytic acid that is hydrolyzed. The Enzyme Nomenclature
Committee of the International Union of Biochemistry recognizes three types of phytases
based on the position of the first phosphate hydrolyzed, those are 3-phytase (EC 3.1.3.8), 4-
phytase (EC 3.1.3.26), and 5-phytase (EC 3.1.3.72). To date, most of the known phytases are
3-phytases or 4-phytases, only a HAP purified from lily pollen and a PTP-like phytase from
Selenomonas ruminantium subsp. lactilytica have been determined to be 5-
phytases.</h4></div>
```

A 2.2 Sample PHP code used for search:

```
<?php error_reporting (E_ALL ^ E_NOTICE);
$var1 = @$_GET['pdbs'];
if($var1=="ids"){
   $var = @$_GET['pdbks'] ;
  $trimmed = trim($var);
$limit=10;
if ($trimmed == "")
  {
echo "<p style=\"margin-left:30px;\">Please enter a search...</p>";
  exit;
 }
if (!isset($var))
  {
echo "<p style=\"margin-left:30px;\">We dont seem to have a search parameter!</p>";
  exit;
}
mysql_connect("localhost","root","");
mysql_select_db("tyrosinase") or die("Unable to select database");
$query = "select * from sequence  where genbankid like \"%$trimmed%\"
  order by genbankid"; // EDIT HERE and specify your table and field names for the SQL
query
 $numresults=mysql_query($query);
 $numrows=mysql_num_rows($numresults);
```

```
if ($numrows == 0)
  {
  echo "<h4 style=\"margin-left:30px;\">Results</h4>";
  echo "<p style=\"margin-left:30px;\">Sorry, your search: &quot;" . $trimmed . "&quot;
returned zero results</p>";
echo "<p style=\"margin-left:30px;\"><a href=\"http://www.google.com/search?pdbks="
  . $trimmed . "\" target=\"_blank\" title=\"Look up
  " . $trimmed . " on Google\">Click here</a> to try the
  search on google</p>";
  }
  else{
  $s=$_GET['s'];
  if (empty($s)) {
  $s=0;  }
  $query .= " limit $s,$limit";
  $result = mysql_query($query) or die("Couldn't execute query");
echo "<p style=\"margin-left:30px;\">You searched for: &quot;" . $var . "&quot;</p>";
echo "<h4 style=\"margin-left:30px;\">Found $numrows Results</h4>";
$count = 1 + $s ;
  while ($row= mysql_fetch_array($result)) {
  $title = $row["keyword"];
  echo "<h5 style=\"margin-left:30px;\">$count) $title</h5>" ;
  $count++ ;
  }
```

```
$currPage = (($s/$limit) + 1);
  echo "<br />";
  if ($s>=1) { // bypass PREV link if s is 0
  $prevs=($s-$limit);
  print " <a style=\"margin-left:30px;\"
href=\"{$_SERVER['PHP_SELF']}?s=$prevs&pdbs=$var1&pdbks=$var\">&lt;&lt;
  Prev 10</a>  ";
}
  $pages=intval($numrows/$limit);
  if ($numrows%$limit) {
    $pages++;
  }
  if (!(($s+$limit)/$limit)==$pages) && $pages!=1) {
  $next=$s+$limit;
 echo " <a style=\"margin-left:30px;\"
href=\"{$_SERVER['PHP_SELF']}?s=$next&pdbs=$var1&pdbks=$var\">Next 10
&gt;&gt;</a>";
  }
$a = $s + ($limit) ;
  if ($a > $numrows) { $a = $numrows ; }
  $b = $s+1 ;
  echo "<p style=\"margin-left:30px;\">Showing results $b to $a of $numrows</p>";
  }
```

A 2.3 Source code of CSS document:

```
/* CSS Document */
body{ background:url(../images/bg1.jpg) repeat-y; background-size:100%;}
div#cointainer{width:950px; margin: 0 auto;}
div#img{ background-image:url(../images/a2.gif); background-repeat:no-repeat;}
div#content{ background-image:url(../images/titbr3.jpg); margin-top:5px; height:26px;
float:left; width:950px; background-repeat:no-repeat;}
div.tab1{float:left; width:150px;; margin-top:5px; background-image:url(../images/layout.gif);
background-repeat:no-repeat; display:inline; }
div.tab1.center{background-image:none; background-color:#DFFFFF; width:620px; margin-
left:15px; margin-right:15px; text-align:justify;}
a:link { COLOR: black; TEXT-DECORATION: none; font-family:Arial, Helvetica, sans-serif
}a:visited { COLOR: black; TEXT-DECORATION: none;font-family:Arial, Helvetica, sans-
serif }
a:active { COLOR: black; TEXT-DECORATION: none }
a:hover { COLOR: blue; TEXT-DECORATION: none; font-family:Arial, Helvetica, sans-
serif }
p.a4{color:#003300; font-size:18px; margin-top:60px; margin-left:200px;}
form{ color:#000066; margin-left:120px;}form.a5{margin-left:120px;}
div.tab1.source{background-image:url(../images/centers.jpg); width:620px; height:450px;
margin-left:15px; margin-right:15px; text-align:justify;}
a.key:link { COLOR: blue; TEXT-DECORATION:underline; font-family:Arial, Helvetica,
sans-serif; }
table td{word-wrap:break-word; word-spacing:inherit;}p.test{width:600px; border:0px;word-
wrap:break-word;}
div.str{ float:left; border:solid; border-width:thin; border-color:#000000; float:left; margin-
left:20px; background-color:#FFFFFF; width:160px; }
```

A

```
/*CSS code for drop down sliding display:*/
body{
        font-family: Trebuchet MS, Lucida Sans Unicode, Arial, sans-serif;
        margin:0px;}
.display_full{
        color:#FFF;font-size:0.9em;
        background-color:none;
        width:430px;margin-bottom:2px;
        margin-top:2px;padding-left:2px;
        background-repeat:no-repeat;
        background-position:top right;
        height:20px;overflow:hidden;cursor:pointer;
}
.display_citation{
        background-color:#FFFFFF;
        border:1px solid #317082;
        width:375px;visibility:hidden;height:0px;
        overflow:hidden;position:relative;
}
.display_citation_content{
        padding:1px;font-size:0.9em;
        position:relative;}
```

```
false;">View in JMOL</a></p>
```

A 2.5 AJAX source code used for source search:

```
//The following code is inside head tag of the document
<script type="text/javascript">

if (window.XMLHttpRequest)
  {// code for IE7+, Firefox, Chrome, Opera, Safari
  xmlhttp=new XMLHttpRequest();

}
else{
xmlhttp=new ActiveXObject("Microsoft.XMLHTTP");
  }
xmlhttp.onreadystatechange=function(){
  if (xmlhttp.readyState==4 && xmlhttp.status==200) {
    document.getElementById("txtHint").innerHTML=xmlhttp.responseText;
    } }
xmlhttp.open("GET","sourcesearch.php?q="+str,true);
xmlhttp.send();}
</script>
// The following code is inside body tag of the document
<p style="margin-left:30px; margin-top:20px;"><b>Source Search</b></p>
<form style="margin-top:30px; margin-left:30px;">
<select name="users" onchange="showUser(this.value)">
<option value="">Select a source:</option><option value="1">Mushroom </option> <option
        value="2">Potato</option><option value="3">Agaricus Bisporus</option>
</select>
</form>
```

A 2.6 Sample source code of sources result page:

```
<div style="margin-left:35px; margin-right:35px; table-layout:auto;">
<?php
$src=$_GET["q"];
$con = mysql_connect("localhost","root","");
if (!$con)  {
  die('Could not connect: ' . mysql_error());
  }
  if($src=="1"){
$file=fopen("src1.html","r");
while(!feof($file)){
  echo fgets($file); }
fclose($file);
}
else if($src=="2"){
$file=fopen("src2.html","r");
while(!feof($file)) {
  echo fgets($file);
  }
fclose($file);
}
mysql_close($con);
?> </div>
```

A

2.7 Sample source code for drop down sliding display (JavaScript):

```
<script type="text/javascript">
var display_slideSpeed = 100;
var display_timer = 10;
var objectIdToSlideDown = false;
var display_activeId = false;
var display_slideInProgress = false;
var display_slideInProgress = false;
var display_expandMultiple = false;
function showHideContent(e,inputId)
{       if(display_slideInProgress)return;
        display_slideInProgress = true;
        if(!inputId)inputId = this.id;
        inputId = inputId + '';
        var numericId = inputId.replace(/[^0-9]/g,'');
        var answerDiv = document.getElementById('display_a' + numericId);
        objectIdToSlideDown = false;
        if(!answerDiv.style.display || answerDiv.style.display=='none'){
        if(display_activeId &&  display_activeId!=numericId && !display_expandMultiple){
        objectIdToSlideDown = numericId;
        slideContent(display_activeId,(display_slideSpeed*-1));
        }else{
        answerDiv.style.display='block';
        answerDiv.style.visibility = 'visible';

                        slideContent(numericId,display_slideSpeed);
                }
        }else{
                slideContent(numericId,(display_slideSpeed*-1));
                display_activeId = false;
        }
}
        slideContent(numericId,display_slideSpeed);
                }
        }else{
                slideContent(numericId,(display_slideSpeed*-1));
                display_activeId = false;
        }}
function slideContent(inputId,direction)
        {
                var obj =document.getElementById('display_a' + inputId);
                var contentObj = document.getElementById('display_ac' + inputId);
                height = obj.clientHeight;
                if(height==0)height = obj.offsetHeight;
                height = height + direction;
                rerunFunction = true;
                if(height>contentObj.offsetHeight){
                        height = contentObj.offsetHeight;
                        rerunFunction = false;}
                if(height<=1){
                        height = 1;
                        rerunFunction = false;}
obj.style.height = height + 'px';
        var topPos = height - contentObj.offsetHeight;
        if(topPos>0)topPos=0;
        contentObj.style.top = topPos + 'px';
```

```
if(rerunFunction){
                setTimeout('slideContent(' + inputId + ',' + direction + ')',display_timer);
        }else{
                if(height<=1){
                        obj.style.display='none';
                        if(objectIdToSlideDown && objectIdToSlideDown!=inputId){
                                document.getElementById('display_a' +
objectIdToSlideDown).style.display='block';
                                document.getElementById('display_a' +
objectIdToSlideDown).style.visibility='visible';
                                slideContent(objectIdToSlideDown,display_slideSpeed);
                        }else{
                                display_slideInProgress = false;
                        }
                }else{
                        display_activeId = inputId;
                        display_slideInProgress = false;
                }}}
function initShowHideDivs()
{       var divs = document.getElementsByTagName('DIV');
        var divCounter = 1;
        for(var no=0;no<divs.length;no++){
        if(divs[no].className=='display_full'){
                        divs[no].onclick = showHideContent;
                        divs[no].id = 'display_q'+divCounter;
```

```
var answer = divs[no].nextSibling;
                        while(answer && answer.tagName!='DIV'){
                                answer = answer.nextSibling;
                        }
                        answer.id = 'display_a'+divCounter;
                        contentDiv = answer.getElementsByTagName('DIV')[0];
                        contentDiv.style.top = 0 - contentDiv.offsetHeight + 'px';
                        contentDiv.className='display_citation_content';
                        contentDiv.id = 'display_ac' + divCounter;
                        answer.style.display='none';
                        answer.style.height='1px';
                        divCounter++;
                }
        }}
window.onload = initShowHideDivs;
</script>
//The following code is inside body dag of the document
<div class="display_full"><a style="color:#0000FF; text-decoration:underline" href="#"
onclick="return false;">Display/Hide Full Abstract</a></div>
<div class="display_citation">
        <div>
//Content
</div>
</div>
```

REFERENCES

Anno, T., Nakanishi, K., Matsuno, R., Kamikubo, T. (1985) 'Enzymation elimination of phytate in soybean milk', J. Japan Soc. Food Sci. Technol.,Vol.32, pp.174-180.

Bitar, K. and Reinhold, J. G. (1972) 'Phytase and alkaline phosphatase activities in intestinal mucose of rat, chicken, calf, and man', Biochim. Biophys. Acta.,Vol. 268, pp.442-452.

Chi, H., Tiller, G. E., Dasouki, M. J. (1999) ' Multiple inositol polyphosphate phosphatase: evolution as a distinct group within the histidine phosphatase family and chromosomal localization of the human and mouse genes to chromosomes 10q23 and 19', Genomics.,Vol. 56, pp.324-336.

Copper, J. R. and Gowing, H. S. (1983) ' Mammalian small intestine phytase', Br. J. Nutr., Vol. 50, pp.673-678.

Craxton, A., Caffrey, J. J., Burkhart, W., Safrany, S. T. and Shears, S. B. (1997) 'Molecular cloning and expression of a rat hepatic multiple inositol polyphosphate phosphatase', Biochem. J.,Vol. 328, pp. 75-81.

Cromwell, G. L., Stahly, T. S., Coffey, R. D., Monegue, H. J. and Randolph, J. H. (1993) 'Efficacy of phytase in improving the bioavailability of phosphorus in soybean meal and corn-soybean meal diets for pigs', J. Animal Sci.,Vol.71, pp.1831-1840.

Dalibor, D., Dvorski, (March 2007) 'Installing, Configuring, and Developing With Xampp', Skills Canada – Ontario. 6th Feb 2012. http://dalibor.dvorski.net /downloads/docs/ InstallingConfiguringDevelopingWithXAMPP.pdf.

David C. Cantu, Yingfei Chen, Matthew L. Lemons and Peter J. Reilly, (2010) 'ThYme: a database for thio ester-active enzymes', Nucleic Acids Research,pp.1–5.

Dennis A., Benson, Mark Boguski, David J., Lipman and James Ostell, (1996)'GenBank', Acids Research,Vol. 24, No. 1.

Dominique Koua, Lorenzo Cerutti, Laurent Falquet, Christian J. A., Sigrist,Gregory Theiler, Nicolas Hulo and Christophe Dunand,(2009)'PeroxiBase: a database with new tools forperoxidase family classification',Nucleic Acids Research,Vol. 37.

Dvorakova, J. (1998) 'Phytase: Sources, Preparation and Exploitation', Folia Microbial., Vol.43, pp.323-338.

Egon Willighagen and Miguel Howard (2005) 'Jmol as 3D Viewer for CDK', CDK News, Vol 2, No.1, pp.17-20.

Ewan Birney and Michele Clamp (2003) 'Biological database design and implementation', Briefings in Bioinformatics,Vol.5, no.1, pp.31-38.

Freund, W. D., Mayr, G. W., Tietz, C. and Schultz, J. E. (1992) 'Metabolism of inositol phosphates in the protozoan Paramecium', Eur. J. Biochem.,Vol. 207, pp. 359 367.

Greaves, M. P., Anderson, G. and Webley, D. M. (1967) 'The hydrolysis of inositol phosphates by *Aerobacter aerogenes*', Biochim. Biophys. Acta.,Vol.132, pp. 412-418.

Greiner, R., Konietzny, U. and Jany, K. D. (1993) 'Purification and characterization of two phytases from *Escherichia coli*', Arch. Biochem. Biophys,Vol. 303, pp.107-113.

Greiner, R. and Konietzny, U. (1996) 'Construction of bioreactor to produce special breakdown products of phytate', J. Biotechnol.,Vol. 48, pp.153-159.

Howson, S. J. and Davis, R. J. (1983) 'Production of phytate-hydrolysing enzyme by fungi.Enzyme Microbiol', Technol., Vol. 5, pp.377-382.

Irving, G. C. J. and Cosgrove, D. J. (1971) 'Inositol phosphate phosphatase of microbial origin. Observations on the nature of the active center of a bacterial (*Pseudomonas sp.*) phytase', Austral. J. Biol. Sci.,Vol. 24, pp.1559-1564.

Jongbloed, A. W., Mroz, Z. and Kemme, P. A. (1992) 'The effect of supplementary *Aspergillus niger* phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of the alimentary tract', J. Animal Sci., Vol.70, pp.1159-1168.

Kim, Y. O., Kim, H. K., Bae, K. S., Yu, J. H. and Oh, T. K. (1998a) 'Purification and properties of a thermostable phytase from *Bacillus sp.* DS11', Enzyme Microbiol Technol.,Vol.22, pp.2-7.

Kim, D. H, Oh, B. C., Choi, W. C., Lee, J. K and Oh, T. K. (1999a) 'Enzymatic evaluation of *Bacillus amyloliquefaciens* phytase as a feed additive', Biotech. Lett.,Vol. 21, pp.925-927.

Laboure, A. M., Gagnon, J. and Lescure, A. M. (1993) 'Purification and characterization of a phytase (myo-inositol hexakisphosphate phosphohydrolase) accumulated in maize (Zea mays) seedlings during germination', Biochem. J.,Vol. 295, pp.413-419.

Lambrechts, C., Boze, H., Moulin, G., Galzy, P. (1992) 'Utilization of phytate by some yeast', Biotechnol. Lett.Vol. 14, pp.61-66.

Laumen, K. and Ghisalba, O. (1994) 'Preparative scale chemo-enzymatic synthesis of optically pure D-myo-inositol 1-phosphate', Biosci. Biotech. Biochem.,Vol. 58, pp.2046-2049.

Liu, B. L., Rafiq, A., Tzeng, Y. M. and Rob, A. (1998). 'The induction and characterization of phytase and beyond', Enzyme Microbiol. Technol.,Vol. 22, pp.415-424.

Maugenest, S., Martinez, I. and Lescure, A. M. (1997) 'Cloning and characterization of a cDNA encoding maize seedling phytase', Biochem. J.,Vol.322, pp.511-517.

Nayini, N. R. and Markakis, P. (1984) 'The phytase of yeast. Food Sci. Technol.,Vol.17, pp.126-132.

O'Quinn, P. R., Knabe, D. A. and Gregg, E. J. (1997) 'Efficacy of Natuphos in sorghum- based diets of finishing swine', J. Animal Sci.,Vol. 75, pp.1299-1307.

Powar, V. K. and Jagannathan, V. (1982) 'Purification and properties of phytate-specific phosphatase from *Bacillus subtilis*', J. Bacteriol.,Vol.151, pp.1102-1108.

Quax, W. J. (1997) 'Merits of secretion of heterologous proteins from industrial microorganism', Folia Microbiol. (Praha),Vol. 42, pp.99-103.

Richardson, N. L., Higgs, D. A., Beames, R. M. and McBride, J. R. (1985) 'Influence of dietary calcium, phosphorus, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile chinook salmon (Oncorhynchus tshawytscha)', J. Nutr.,Vol.115, pp.553-567.

Sandberg, A. S., Hulthen, L. R. and Turk, M. (1996) 'Dietary *Aspergillus niger* phytase increases iron absorption in humans', J. Nutr.,Vol.126, pp.476-480.

Satyajeet, P. Khare, Farhat Habib2, Rahul Sharma, Nikhil Gadewal, Sanjay Gupta,and Sanjeev Galande, (2011) 'HIstome—a relational knowledgebase of human histone proteins and histone modifying enzymes', Nucleic Acids Research,Vol.1 pp.1–6

Shah, V. and Parekh, L. J. (1990) 'Phytase from Klebsiella sp. No. PG-2: Purification and properties', Indian J. Biochem. Biophys,Vol.27, pp.98-102.

Shieh, T. R. and Ware, J. H. (1968) 'Survey of microorganisms for the production of extracellular phytase', Appl. Microbiol.,Vol.16, pp.1348-1351.

Shimizu, M. (1992) 'Purification and characterization of phytase from *Bacillus subtilis* (natto) N-77', Biosci. Biotechnol. Biochem.,Vol.56, pp.1266-1269.

Shobana Sugumari and Berla Thangam (2012) 'BiodEnz:A database of biodegrading enzymes',Bioinformation,Vol.1,No.1,pp.40-42.

Ullah, A. H. J. and Phillippy, B. Q. (1988) 'Immobilization of *Aspergillus ficuum* phytase:product characterization of the reactor', Prep. Biochem., Vol.18, pp.483-489.

Volfova, O., Dvorakova, J., Hanzlikova, A. and Jandera, A. (1994) 'Phytase from *Aspergillus niger*', Folia Microbiol.,Vol. 39, pp.481-484.

Wendy Baker, Alexandra van den Broek, Evelyn Camon, Pascal Hingamp,Peter Sterk, Guenter Stoesser and Mary Ann Tuli, 'The EMBL Necluotide Sequence Database',Nucleic Acids Research.,Vol.28.

Williams, P. J. and Taylor, T. G. (1985) 'A comparative study of phytate hydrolysis in the gastrointestinal track of the golden hamster (*Mesocricetus auratus*) and the laboratory rat', Br. J. Nutr.,Vol. 54, pp.429-435.

Wodzinski, R. J., and Ullah, A. H. J., (1996) 'Phytase. Adv. Appl', Microbiol,Vol. 42, pp.263-302.

Wolstencroft, K. J., Stevens, R., Tabernero, L., and Brass, A., (2005) 'PhosphaBase: An Ontology-Driven Database Resource for Protein Phosphatases ', PROTEINS: Structure, Function, and Bioinformatics.,Vol. 58, pp.290–294.

Wyss, M., Brugger, R., Kronenberger, A., Remy, R., Fimbel, R., Oesterhelt, G., Lehmann, M. and van Loon, A. P. G. M. (1999b) 'Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases) catalytic properties, Appl', Environ. Microbial,Vol. 65, pp.367-373.

Yang, W. J., Matsuda, Y. Sano, S., Masutani, H. and Nakagawa (1991a) 'Purification and characterization of phytase from rat intestinal mucosa', Biochim. Biophys Acta.Vol.1075, pp.75-82.

Yi, Z., Kornegay, E. T., Ravindran, V., and Denbow, D. M. (1996) 'Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorus equivalency values for phytase'. Poultry Sci.,Vol.75, pp.240-249.

Yoon, S. J., Choi, Y. J., Min, H. K., Cho, K. K., Kim, J. W., Lee, S. C. and Jung, Y. H. (1996) 'Isolation and identification of phytase-producing bacterium, *Enterobacter sp*. 4, and enzymatic properties of phytase enzyme', Enzyme Microbiol. Technol.,Vol.18, pp.449-454.