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Research article

Computational based functional analysis of *Bacillus* phytases

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ABSTRACT

Phytase is an enzyme which catalyzes the total hydrolysis of phytate to less phosphorylated myo-inositol derivatives and inorganic phosphate and digests the undigestable phytate part present in seeds and grains and therefore provides digestible phosphorus, calcium and other mineral nutrients. Phytases are frequently added to the feed of monogastric animals so that bioavailability of phytic acid-bound phosphate increases, ultimately enhancing the nutritional value of diets. The *Bacillus* phytase is very suitable to be used in animal feed because of its optimum pH with excellent thermal stability. Present study is aimed to perform an *in silico* comparative characterization and functional analysis of phytases from *Bacillus amyloliquefaciens* to explore physico-chemical properties using various bio-computational tools. All proteins are acidic and thermostable and can be used as suitable candidates in the feed industry.

1. Introduction

Over the years the need for a phosphatase enzyme has escalated due to the complications being caused by phytic acid. Phytic acid (myo-inositol hexakisphosphate or phytate) is found in beans, seeds, nuts, grains, tubers, cereals, legumes, oilseeds and in certain fruits and vegetables like berries and green beans and is the principal storage form of phosphorus. It triggers weakened absorption of mineral ions like zinc, calcium, iron, magnesium and copper and also inhibits certain digestive enzymes like pepsin, trypsin and amylase that can lead to poor bone growth, short stature, rickets, narrow jaws, anemia, tooth decay and mental retardation in human beings. Phytic acid can severely hinder nutrient absorption by intestine (Lopez et al., 2002; Konietzny and Greiner, 2002; Kumar et al., 2010; Afinah et al., 2010)

One such enzyme is phytase (myo-inositol hexakisphosphate phosphohydrolase) that hydrolyzes phosphate residues from phytic acid. It hydrolytically cleaves free bound phosphorus (inorganic and undigestable form) from phytic acid molecule releasing phosphorus, calcium and magnesium cations (organic and digestable form). Phytase is a monomeric enzyme that has molecular mass between 40 to 70 kDa (Konietzny and Greiner, 2002; Kumar et al., 2010; Afinah et al., 2010; Greiner and Konietzny, 2006).

Phytase activity has been reported in various plant and animal tissues like in rice, maize, legume seeds, soybean, barley and in rat (intestine and liver) tissue. It is also found in numerous micro-organisms like bacteria (*Bacillus* sp., *Eschirichia coli, Pseudomonas* sp.), fungi (*Aspergillus* sp., *Rhizopus* sp., *Penicillium* sp.), yeast (*Saccharomyces* sp., *Candida* sp.) and other related species (Konietzny and Greiner, 2002; Kumar et al., 2010; Afinah et al., 2010). There are several classes of phytase that are based on various factors like the site where hydrolytic activity is commenced which includes the 3-phytase that liberates P moiety at position C3 and 6 phytase that releases it at position C6 of the hexaphosphate ring. Another class is grounded on the basis of pH activity which comprises of histidine acid phosphatases that show optimum activity at pH 5 and alkaline phosphatases that indicate ideal activity at pH 8. Several other are the beta propeller phytase, purple acid phosphatases and the very rare protein tyrosine phosphatase like phytases (Kumar et al., 2010; Greiner and Konietzny, 2006; Kerovuo et al., 1998).

Phytase has been recognized to show tremendous potential in the industry. Phytase is employed in bakery as a bread making improver to refine its volume, softness and shape. Phytase enhances digestibility of phytate associated phosphorus and is thus applied in the animal feed. It increases phosphorus activity and so exercised as a transgenic crop. Due to its degrading property it is utilized in pulp and paper industry and as an antioxidant in food products to increase the nutritional value, flavour, colour and to decrease microbial spoilage. It also has an application as a therapeutic against cancer, heart related and dental problems. Technical improvements have also been observed in corn wet milling and fractionation of cereal bran (Kumar et al., 2010; Afinah et al., 2010; Greiner and Konietzny, 2006) Different classes and

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sources of phytase show diverse properties and thus show a range of functions in the industry.

This study incorporates the use of various bio-computational tools to perform the structural and functional analysis of *Bacillus amyloliquefaciens* containing the phytase enzyme in order to decipher its inimitable properties concomitant to its industrial application.

2. Materials and methods

2.1. Sequence retrieval

Swiss prot/UniProtKB server that is the knowledge base of SIB (Swiss Institute of Bioinformatics) and main proteomics based resource server expasy (expert protein analysis system) (Artimo et al., 2012; Apweiler et al., 2004) (http://www.expasy.org/) were used for protein sequence retrieval of *Bacillus*(gram positive, rod shaped bacteria) species having phytase activity.

Further full length phytase protein sequences were retrieved for *in silico* study. Total 13 proteins sequences of different strains of *Bacillus amyloliquefaciens* were downloaded in FASTA format for computational investigation.

2.2. Physicochemical characterization

A java based Akriti v1.0 software (www.insilicogenomics.in/mfpcalc/mfpcalc.html) server was used to calculate the physicochemical properties of retrieved sequences. Amino acid composition, pI or isoelectric point (pH at which net charge is zero), extinction coefficient (quantitative study of protein–protein and protein–ligand interactions), instability index (stability of proteins), aliphatic index (relative volume of protein occupied by aliphatic side chains), GRAVY or Grand Average Hydropathy (sum of all hydropathy values of all amino acids divided by number of residues in a sequence) were analyzed using Akriti v1.0 software.

2.3. Secondary structure prediction

Secondary structure consisting secondary elements i.e. helix, turn and sheet were predicted using PSIPRED and CFSSP: Chou and Fasman Secondary Structure Prediction server (http://www.biogem.org/cgi-bin/cho-fas.pl) (McGuffin et al., 2000; Kumar, 2013). These secondary structures combine to form the tertiary (3D) structure that determines the function of a protein.

2.4. Functional analysis

For functional analysis CYS_REC was used to identify position of cystine and compute most probable SS bond pattern of pairs in protein (Hooda, 2011). The set of conserved amino acid residues were analyzed using Motif search tool (http://www.genome.jp/tools/motif/) (Singh et al., 2012) and membrane and soluble proteins were distinguished using SOSUI server that was able to predict transmembrane helices from amino acid sequences with high precision and accuracy (Suwa et al., 2011).

2.5. Structural classification

For structural classification CATH server was used (http://www.biochem.ucl.ac.uk/bsm/cath). CATH database groups the protein structures on the basis of similarity or a common evolutionary origin using manual curation along with certain algorithms. This classification procedure includes the distribution of proteins into four groups that are (C) lass, (A)rchitecture, (T)opology and (H) omologous family. (Sillitoe et al., 2013).

3. Result and discussion

3.1. Sequence retrieval

Total 10 phytase sequences were retrieved for different *Bacillus* species (*Bacillus methylotrophicus, Bacillus siamensis, Bacillus subtilis, Bacillus licheniformis, Bacillus pseudomycoides, Bacillus mycoides, Bacillus cereus, Bacillus atrophaeus, Bacillus coagulans*) along with *B. amyloliquefaciens*. After phylogenetic classification it has been investigated that *B. amyloliquefaciens*is closer to *B. methylotrophicus, B. siamensis, B. subtilis, B. licheniformis, B. pseudomycoides, B. mycoides, B. atrophaeus, B. coagulans* and differ with *B. cereus*(Fig. 1). Based on the imminence to other strains and the industrial prominence of *B. amyloliquefaciens*, 13 full length phytase proteins were retrieved for different strains of *B. amyloliquefaciens* that were available in uniprot with equal number of amino acids (length) to show homogeneity in our study (Table 1). To check the evolutionary relationship and diversity of selected proteins multiple sequence analysis and tree construction were performed. Further these sequences were used for physiochemical characterization to identify the unique properties that makes them suitable to be used as feed additives and to select the most suitable candidate (Schallmey et al., 2004). The phylogeny of selected 13 strains of *B. amyloliquefaciens* were shown in Fig. 2. This

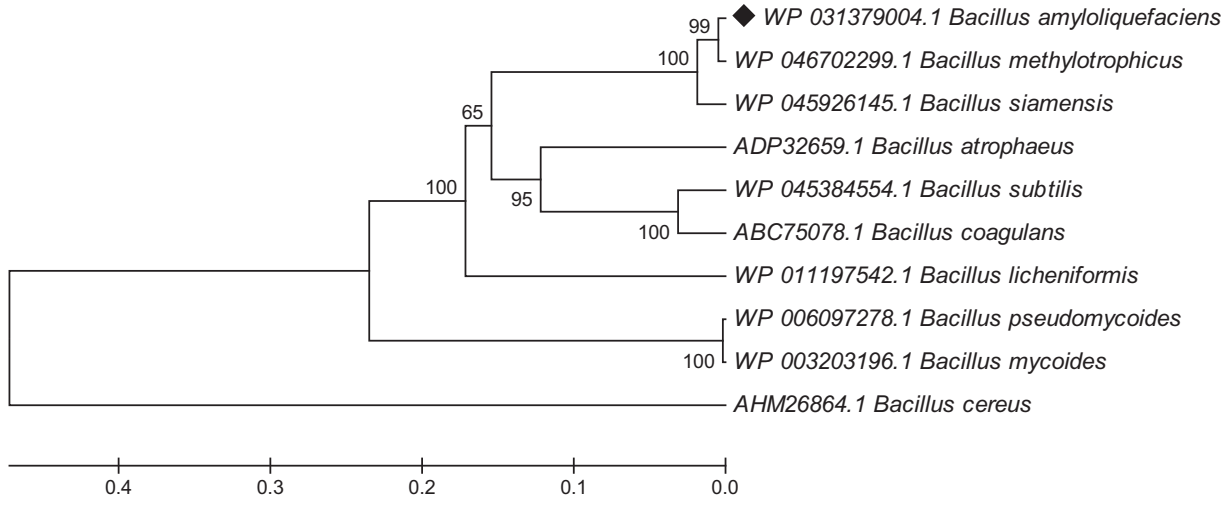

Fig. 1. Phylogenetic classification of 10 phytase proteins from different *Bacillus* sp.