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Role of phytase producing microorganisms towards agricultural sustainability

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ABSTRACT

Focusing towards sustainable and environment friendly agricultural techniques, phytase enzymes have proved to be beneficial in many aspects. It is a group of enzymes, which are capable of degrading phosphate containing organic molecules, henceforth release inorganic phosphorus in a stepwise reaction. Studies have shown that soil contain phosphorus mainly in the form of an organic compound, phytate, which cannot be directly utilized by plants and can cause heavy contamination. Hence, phytases are exogenously added which modify these molecules such that their uptake can enhance the growth of plants and provide appropriate nutrition. Numerous phytases have now been characterized from different sources and employed to protein engineering techniques, bioremediation of phytate-contaminated soil, animal nutrition and in food processing for humans, which has now included the enzyme in the category of nutraceuticals. Formulations in phytase enzyme have helped in achieving good stability during storage, high bioefficacy, and dust freeness.

1. Introduction

Phytases [*myo*-inositol (1,2,3,4,5,6) hexakisphosphate phosphohydrolases] are a sub-group of phosphatase enzymes, which initiate stepwise dephosphorylation of phytic acid to release inorganic phosphorus (Higgins and Crittenden, 2015). Phytic acid (also known as phytate, when in salt form) is chemically known as *myo*-inositol (1,2,3,4,5,6) hexakisphosphate and is the main storage form of phosphorus in plant seeds and pollen. Phytate is known to be the most abundant form of inositol phosphorus in nature. It is the hexaphosphoric ester of the hexahydric cyclic alcohol meso-inositol wherein phytases sequesters orthophosphate groups from its inositol ring to produce free inorganic phosphorus, along with a chain of intermediate myo-inositol phosphates (Kumar et al., 2012).

Since humans, dogs, pigs, birds, and other non-ruminant animals lack phytase enzyme, phosphorus is not utilized when it is in bound form as phytate, present in plant feeds. It is excreted directly in the areas of intensive animal agriculture, which contribute towards environmental phosphorus pollution that has recently been considered as an important ecological concern. The excessive phosphorus may also cause eutrophication and generation of neurotoxins by stimulating growth of aquatic organisms (Holm et al., 2002; Lei and Porres, 2003). As phytate is a negatively charged ion, it forms complexes with divalent and trivalent mineral cations present in plant-based food products (like Cu^{2+}, Zn^{2+}, and Cd^{2+}) and reduces their bioavailability. They form insoluble phytate-mineral complexes that render mineral ions unavailable for absorption by gastrointestinal tract of humans and hence remain partially hydrolyzed in human gut (Lopez et al., 2002; Mittal et al., 2013). Phytate has also shown a negative impact by forming strong complexes with proteins, which are less likely to be degraded by proteolytic enzymes, thereby inhibiting their proteolysis and consequently affecting amino acids digestion in humans. Due to the presence of charged phosphate groups on phytate, it exhibits chelating capacity and subsequently forms binary complexes (phytate-protein) or ternary complexes (phytate-mineral-protein). This affects the hydrolysis of phytate and release of amino acids in proteins from phytate (Kong and Adeola, 2011). Since lipids and carbohydrates are also positively charged, phytate forms complexes with these molecules and inhibits their uptake. Hence, being apprehensive of the severity of phytate levels in food products, there is no way to annul its side effects. As a solution, phytase research and its applications are in vogue for degrading antinutrient phytate during food processing and in agriculture to maintain sustainability (Kumar et al., 2010).

The mechanism of action of all described phytases is based on the enzymatic hydrolysis of the bonds between inositol and phosphoric acid residues. An enzymatic hydrolysis of bonds occurs between inositol and phosphoric acid residues upon which the mechanism of action of all phytases is based. The end-products of this series of reactions are 6-fold alcohol and phosphates (Mukhametzyanova et al., 2012). Fresh plant residues are decomposed by microbial phytases in the soil leading to the

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release of phosphorus from organic compounds. There are different orders along with different rates of reactions by which the phosphoric acid residues are released by the process of microbial hydrolysis of phytate (Mukhametzyanova et al., 2012). The histidine acidic phytases catalyze the release of phosphates adjacent to free hydroxyl group, after the dephosphorylation of first phosphate group. Generally, the plant phytases exhibit difference in intermediate myo-inositol product pentaphosphate formation during the first stage of reaction. During the first step of hydrolysis, microbial 6-phytases form a different set of intermediates. In Enterobacteriaceae, acid phosphatases having phytate hydrolyzing properties have been reported to hydrolyze glucose-1-phosphate and the phosphate residue of phytate molecule in D-3 position is cleaved (Greiner and Sajidan, 2008). Myo-inositol triphosphates are formed as end products of the reaction by alkaline phosphatases in lily pollen, *B. subtilis* and macereed (Greiner, 2007; Greiner and Sajidan, 2008; Mukhametzyanova et al., 2012).

2. Classification of phytases

In the past two decades, extensive research has been carried out in order to isolate and categorize phytases from different sources. They are grouped according to their catalytic mechanism, pH activity, and the initiation site of dephosphorylation of phytate. Based on the catalytic mechanism, phytases can be classified as histidine acid phytases (HAP) (EC 3.1.3.2), β-propeller phytases (BPP) (EC 3.1.3.8), cysteine phytases or purple acid phytases (PAP) (EC 3.1.3.2) and recently identified protein tyrosine phosphatase (PTP)-like phytases (Ruijuan et al., 2010). The conserved domains of all three phytases are designated as pfam00328, pfam02333, and pfam02227, respectively according to the conserved domain database (CDD) of the NCBI. Histidine acid phytases share an active site sequence (RHGXRXP), the catalytic dipeptide, and 10 cysteine residues. These phytases are mainly from fungi and *E. coli.* β-propeller phytases have a six-bladed propeller-folding configuration (Fig. 1) (Verma et al., 2016). Each protein molecule includes six calcium-binding sites and the enzyme activity is dependent on calcium. They are mainly isolated from *Bacillus* species. Purple acid phosphatases are homodimeric glycoproteins, with Fe (III)–Zn (II) as their active site (Rao et al., 2009). They are isolated from plant species whereas cysteine phytase is isolated from anaerobic ruminal bacterium *Selenomonas ruminantium* (Chu et al., 2004; Jain and Singh, 2016).

According to the pH optima, acid phytases generally include HAP, PAP, and PTP-like phytases. Whereas, alkaline phytases comprises only BPPs from *Bacillus* species (Singh and Satyanarayana, 2015; Tye et al., 2002). On the other hand, based on the carbon position at which dephosphorylation is initiated; phytases are grouped into 3-phytase (myo-inositolhexakisphosphate 3-phosphohydrolase), 6-phytase (*myo*-inositolhexakisphosphate 6-phosphohydrolase) and 5-phytase (myo-inositolhexakisphosphate 5-phosphohydrolase). 3-phytase (E.C.3.1.3.8) initiate hydrolysis of phytate at third position group, and are isolated from *Aspergillus niger*, *Neurospora crassa*, *Pseudomonas*, and *Klebsiella* sp. ASR1. 6-phytase (E.C.3.1.3.26) hydrolyzes at sixth phosphate group and is extracted from *E. coli*, *Paramecium*, and many more, whereas 5-phytase (E.C.3.1.3.72) initiates phytate hydrolysis at the fifth phosphate group and is isolated from *Medicago sativa, Phaseolus vulgaris, and Pisum sativum*. Significant differences between dephosphorylation steps of phytate by 3-phytases and 6-phytases have marked the former to be better than the latter. This is because of the end product that 3-phytases form, which is IP_1, upon continuous degradation of phytate. On the other hand, 6-phytases halts their action upon the formation of IP_4 and other lower esters that give less efficiency at commercial level (Greiner and Konietzny, 2011).

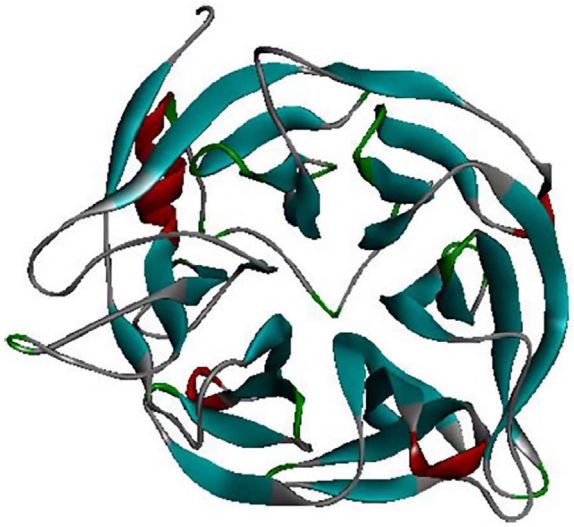

Fig. 1. Three dimensional structure of *Bacillus amyloliquefaciens* phytase (Verma et al., 2016).

3. Development of phytase from different sources

As the first scientific reference of phytic acid came out in literature, Suzuki and his co-workers initiated research on phytate degrading enzymes and isolated phytase from rice bran just after four years of phytate discovery (Suzuki et al., 1907). Since then many phytate sources were identified like microbial phytase, including bacterial and fungal sources and also plant phytase, some of which are listed in Table 1.

3.1. Plant phytase

Most of the phytases isolated from plant sources are of type 6-phytases as they initiate hydrolysis at sixth carbon position of the myo-inositol hexaphosphate ring. Successful dephytinisation is observed during germination through endogenous enzymatic activity but it has been proved as highly species dependent, like maize, millet and sorghum initially exhibit low phytase activity, which increases rapidly after germination. On the other hand, wheat, barley and rye all have high phytase activity in the grain (Bohn et al., 2008; Gupta et al., 2015). Apart from these, phytase have also been isolated and characterized from rice (Hayakawa et al., 1989), rapeseed (Houde et al., 1990), soybean (Hamada, 1996), pea, barley, bean, potato, radish, spinach and many more (Dvorakova, 1998).

To get more yield of phytase, transgenic plants have been produced with microbial genes expressing phytase. Most of these plants overexpress histidine acid phytase (HAP) (Brinch-Pedersen et al., 2014; Gutkneckt, 1997) and β-propeller phytase (BPP) (Lung et al., 2005; Shen et al., 2016) gene in the host. These experiments focused on commercial production of phytases, like phytase-transgenic tobacco and alfalfa. But the major concern with production of phytases for feed application is its irreversible heat inactivation during pelleting which is performed at temperatures between 65 °C and 95 °C. Hence, to fulfil the quality criteria, the enzymes that are isolated for commercial purposes should be resistant to heat inactivation and cheap to produce (Konietzny and Greiner, 2004). Transgenic rice seeds have been developed for human application by introducing a thermostable phytase from yeast, *Aspergillus fumigatus* into rice endosperm to increase iron bioavailability (Lucca et al., 2001). Other plants in which transgenesis have been carried out are rice, wheat, sugarcane, alfalfa, arabidopsis, sesame, soybean, barley, potato, and canola (Abid et al., 2016; Holme et al., 2016). Since plant roots exudates have less phytase activity, the plant does not utilize phytate efficiently, and due to this inorganic phosphorus sources are added exogenously during plant growth for optimization of nutrients that are available to them. To avoid cost of such sources, one way is the overexpression of phytase in plant roots that will be able to consume soil phytate, thus reducing agricultural