A Review on Role of Microbial Phytases in Agriculture

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Abstract

Phytases are a group of enzymes which hydrolyze phytic acid to less phosphorylated *myo*-inositol derivatives releasing inorganic phosphate. Phytases can be derived from a host of sources like plants, animals and microorganisms. Microbes are more promising for the production of phytases on a commercial scale and thus the production of phytase from microbial origin is of greater potential in development. There are wide applications of phytases – in animal nutrition, in aquaculture, in human nutrition, in human health and in agriculture. The role of phytases in agriculture is discussed in detail in the current review. This review is focused on various aspects of isolation and screening of phytase producing bacteria and testing the efficacy of isolates in promoting plant growth.

KEYWORDS: Phytases, microbial sources, agriculture, plant nutrition

Introduction:

Phytases *(myo*-inositol hexakisphosphate phosphohydrolases) are a group of enzymes which hydrolyze phytic acid to less phosphorylated *myo*-inositol derivatives (in some cases to free *myo*-inositol), releasing inorganic phosphate (Nagai & Funahashi, 1962; Jareonkitmongkol *et al*., 1997; Kim *et al*., 1998; Thanaa H. Ali *et al*., 2007). Phytases or phytate-degrading enzymes belong to a special class of phosphomonoesterases termed *myo*-inositol hexakisphosphate phosphohydrolases, which are capable of initiating the stepwise release of phosphate residues from phytate (Greiner, 2007).The complete hydrolysis of phytate produces one molecule of inositol and six molecules of inorganic phosphate, while partial hydrolysis results in *myo*inositol intermediates, namely, mono-, di-, tri-, tetra-, and pentaphosphates besides inorganic phosphate. Phytases are broadly classified into three types depending on the initiation site of dephosphorylation of the phytate, namely, 3-phytases, 6-phytases, and 5-phytases (Cosgrove, 1970).

There are wide applications of phytases – in **animal nutrition** (Cromwell *et al*., 1995b; Ravindran *et al*., 1995; Sebastian *et al*., 1997; Selle *et al*., 2000; Camden *et al*., 2001; Zyla *et al*., 2004; Onyango *et al*., 2005; Fritts & Waldroup, 2006; McClung *et al*., 2006; Cowieson *et al*., 2006) in **aquaculture** (Robinsin *et al*., 1996; Oliva-Teles *et al*., 1998; Mwachireya *et al*., 1999, Van Weerd *et al*., 1999, Sajjadi & Carter, 2004; Vielma *et al*., 2004; Debnath *et al*., 2005; Baruah *et al*., 2005, 2007; Nwanna & Schwarz, 2007), in **human nutrition** (Sandberg *et al*., 1996; Lonnerdal *et al*., 1988; Greiner & Konietzny, 1998, 1999, 2006; Lucca *et al*., 2001; Haros *et al*., 2001; Palacios *et al*., 2005, 2006, 2008a, 2008b), in **human health** (Claxon *et al*., 1990; Jariwalla *et al*., 1990; Carrington *et al*., 1993; Shears, 1998; Grases *et al*., 2000; Shamsuddin, 2002; Vucenik & Shamsuddin, 2003; Maffucci *et al*., 2005) and in **agriculture**. The aim of this review is to discuss the role of

phytases in agriculture. These phytases can be used for P mineralisation in soil and thus help plant nutrition and thus increase agriculture yields. Several bacteria possessing phytase activities can be employed for phosphorus nutrition in plants and thus used in improving agriculture.

Phytases in Agriculture: Phosphorus is an essential plant nutrient that limits agricultural production on a global scale. Many agricultural soils are deficient in phosphorous (P) readily available for plants and therefore require the application of P-based fertilizers to remain productive (Uzair & Ahmed, 2007). Consequently, fertilized soils contain a significant amount of total soil phosphorous, of which approximately 30–80% is bound in organic form. Major components of soil organic phosphorous are the soil phytates (~50%) mainly consisting of inositol penta- and hexa-phosphates and is poorly utilized by plants (Dalal, 1977; Anderson, 1980; Richardson *et al.,* 2001, 2001b)**.** To be available to plants, organic P substrates must be first hydrolyzed by phosphatase enzymes to release free phosphate (Pi) in soil. The ability of plants to use phosphorus from soil is improved when soil are inoculated with microorganisms that possess the ability to exude phytase, or when a purified phytase is added (Richardson, 1997; Hayes *et al*., 2000; Richardson *et al.,* 2000, 2001b). Phytase ability derived from soil microorganisms such as *Pseudomonas* sp. (Richardson *et al.,* 2000, 2001b) and *Bacillus amyloliquefaciens* (Idriss *et al.,* 2002) has been shown to contribute to plant phosphorous nutrition. Findenegg and Nelemans (1993) studied the effect of phytase on the availability of phosphorus from phytic acid in the soil for maize plants. Growth stimulation was reported as the result of an increased rate of phytin hydrolysis when phytase was added to the soil. However, the large amount of phytase necessary to give a significant effect meant that the technique was not practical at this time.

It was also suggested that expression of phytase in the roots of transgenic plants might increase availability of phosphorus to plant roots (Day, 1996). To better understand the role root phytase plays in phosphorus nutrition of plants, extracts from several temperate pasture grass and legumes containing phytase and acid-phosphatase activity were studied (Hayes *et al.,* 1999). Root phytase has been suggested as one of the mechanisms of plants to improve utilisation of soil phosphate. Due to the low phytase activity in roots and the inability of phytase secretion into the rhizosphere, phytate was poorly utilised by the plants (Hayes *et al.,* 2000). Thus it was suggested, that soil microorganisms colonising the plant rhizosphere and producing extracellular phytase activity, such as *Bacillus* sp. and *Enterobacter* sp. could act as plant growth promoting rhizobacteria (PGPR) by making phytate phosphate available to the plant (Idris *et al.,* 2002). The importance of phosphate availability from soil phytate for plant nutrition under phosphate limitation was demonstrated by enabling the plants to utilise phytate phosphate by addition of purified phytase as well as soil microorganisms like *Aspergillus* expressing extracellular phytase activity to the rooting medium (Richardson *et al*., 2001; Idris *et al*., 2002).

Sources of Phytases: These Phytases are widespread in nature and have been reported in plant and animal tissues and in a variety of microorganisms. Phytase occurs widely in the plant kingdom, in cereals, oilseeds and by-

products, which varies according to grain and type of by-product. In higher plants phytases occur predominantly in grains, seeds and pollen (Reddy *et al*., 1989; Konietzny & Greiner, 2002). Phytase has been found to exist in monogastric animals (Bitar & Reinhold, 1972; Copper & Gowing, 1983; Yang *et al*., 1991a; Chi *et al*., 1999). Although phytases can be derived from a host of sources, microorganisms are more promising for the production of phytases on a commercial scale (Pandey *et al*., 2001a; Nam-Soon Oh & Man-Jin In, 2009). The mass production of phytase from plant and animal origin is not economic since preliminary treatment is necessary and the production procedure becomes time-comsuming, troublesome and expensive. Recent research has shown that microbial phytases are most promising for biotechnological applications because of the capacity of the microorganisms to produce and secrete large quantities of enzymes combined with the desired temperature and pH activities and stability properties (Sharon *et al*., 2008). Therefore, the production of phytase from microbial origin is of greater potential in development. Several fungal, bacterial and yeast strains have been reported as the source of phytase. Some of the phytase producing microorganisms include bacteria such as *Bacillus subtilis* (Powar & Jagannathan,1982), *Escherichia coli* (Greiner R. *et al*.,1993), Yeasts such as *Saccharomyces cerevisiae* (Nayini & Markakis,1984), *Schwannoiomyces castellii* (Lambrechts *et al*., 1992) and fungi such as *Aspergillus niger* (Shieh *et al*., 1969), *A. oryzae* (Shimizu,1993), *A. ficuum* (Ullah & Phillippy,1988) and *Penicillium sp.* (Shieh & Ware,1968). Due to several biological characteristics, such as substrate specificity, resistance to proteolysis and catalytic efficiency, bacterial phytases have considerable potential in commercial applications.

Isolation and screening of phytase producing bacteria: Bacteria are though ubiquitous in their occurrence, the most common sources for their isolation are soils, lakes and river mud. Most phytase producing microorganisms from nature were isolated from soils (Shieh & Ware, 1968; Howson & Davis, 1983; Tseng *et al.*, 2000; Anis Shobirin *et al.*, 2009). The phytases have been isolated from various sources such as maize plantation (Anis Shobirin *et al.*, 2009), from the rhizosphere soil of leguminous plant methi (*Medicago falacata*) (Gulati *et al*., 2006), soil sample of poultry waste dumps (Mukesh *et al.*, 2004). The technique of isolating microorganisms varies according to the nature and physiological properties of the microbe to be isolated. The more classical method to isolate new bacteria is direct isolation on solid media. Enrichment culture is also frequently used in order to isolate microorganisms having special growth characteristics. It allows selective cultivation of one or more bacterial strains obtained from a complex mixture such as that found in most soils. The method typically relies on using a particular compound as the sole carbon source or less frequently as the nitrogen, sulphur or phosphorus sources. The choice of the medium and the conditions used in the enrichment culture favours the growth of the desired forms. The most useful plate technique for screening phytase producing microorganisms is based on the production of clear zones of hydrolysis around the colonies, which later are subjected to fermentation and estimated for phytase activity.

Field studies on use of Phytases for plant nutrition: Field trials need to be made to study the contribution of secreted phytases to the observed plant growth promotion by culture filtrates of various isolates. Experiments should be conducted to study the influence of selected isolates on seed germination, growth (shoot length, root length) and nutrient uptake of various plants (tomato or tobacco or maize plants) under net house conditions.

Conclusion: Studies have to be undertaken to isolate and screen high yielding phytase producing bacteria. The isolates need to be tested for their ability to mineralize phosphate and their use in improving plant nutrition. Thus high yielding phytase producing bacteria can be employed as PGPR (Plant Growth promoting Rhizobacteria) for increasing agriculture yields.

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