Applications of phytase of thermophilic mould, *Sporotrichum thermophile*: A review

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This study presents a review on applications of phytase from thermophilic fungal isolate, *Sporotrichum thermophile*. Under optimization, improvement in phytase production can be achieved in both solid state (2-fold) and submerged (2.6-fold) fermentations. Phytase could also be produced by alginate-immobilized *S. thermophile*, and enzyme production was sustainable over 5 repeated cycles. Purified phytase is a homopentameric glycoprotein (mol mass, 456 kD) and is optimally active at pH 5.0 and 60°C with a T

1/2 of 16 h at 60°C and 90 min at 80°C. It is insensitive to trypsin and pepsin, and unaffected by EDTA. Phytase has all requisite properties for application as a feed and food additive, dephytinization of soymilk and wheat flour and soil conditioner for plant growth promotion.

**Keywords:** Phytase, Phytic acid, Plant growth promotion, *Sporotrichum thermophile*, Thermophilic mould

**Introduction**

Phytic acid is principal storage form of phosphorus (P, 1-5% by wt) in cereals, legumes, oilseeds and nuts1-5. In forage, one-third of P is present as digestible inorganic P, while two-thirds as organic P as phytin, which is a mixture of calcium–magnesium salts of inositol-hexaphosphoric acid, phytic acid. In plant derived foods, phytic acid acts as an anti-nutritional factor, since it causes mineral deficiency due to efficient chelation of metal ions (Ca2+, Mg2+, Zn2+ and Fe2+) forming complexes with proteins and thus affecting their digestion and also inhibits enzymes6,7 (amyrase, trypsin, acid phosphatase and tyrosinase). Phytic acid and inositol phosphate intermediates have been implicated in lowering of cholesterol and triglycerides, in affecting blood glucose response, in tumor development, in treatment of Parkinson’s disease, Alzheimer’s disease and multiple sclerosis5. Phytate P is largely unavailable to monogastric animals due to lack of adequate levels of phytate degrading enzymes in their gastrointestinal tract1-5. Since phytic acid cannot be utilized as source of P, feeds for pigs and poultry are commonly supplemented with inorganic phosphate to meet their P requirement. Supplemented inorganic P and excreted phytate P impose global ecological problems (eutrophication) when it enters into rivers, lakes and other water bodies, resulting in algal blooms, hypoxia and death of marine animals8-9. Due to these problems, there is a considerable interest in phytate-degrading enzymes (phytases), thereby eliminating the ability of phytic acid to chelate metal ions, inhibiting enzymes and forming complexes with proteins. Supplementation of phytase in fodder improves P bioavailability and reduces P excretion in areas of intensive livestock production. Phytases aid in production of special isomers of various lower phosphate esters of myo-inositol, some of which are considered to be pharmacoactive and important intracellular secondary messengers5.

Reduction of phytic acid in foods and feeds by enzymatic hydrolysis using phytase improves nutritional value of foods. Phytase also has potential applications in other fields, and is of immense commercial value primarily in feed and food industries. Undoubtedly, increasing public concern regarding environmental impact of high P levels in animal excreta has driven biotechnological development
of phytase and its application in animal nutrition. Feeding trials have shown effectiveness of supplemental microbial phytases in improving utilization of phytate-P and phytate-bound minerals in swine and poultry. Thermophilic moulds produce phytases.

This review presents production, characteristics and potential biotechnological applications of an extracellular phytase of thermophilic mould, *Sporotrichum thermophile*.

**Screening and Selection of Phytase-Producing Thermophilic Mould**

Thermophilic fungi have been isolated from soils, composts, retting guayule, stored grains, birds and animals excreta and others. Majority of isolates of thermophilic moulds grow on phytase screening medium (PSM) solid plates but only few fungi showed zone of calcium phytate hydrolysis. When these zone forming fungi cultivated on PSM agar plates containing sodium phytate, only few thermophilic moulds showed hydrolysis zone after double staining method, may be due to formation of various acids (acetic acid, malic acid etc.), which solubilize calcium phytate resulting in zone formation. These acids lower medium pH, and hence, increase solubility of calcium phytate. Number of fungi producing phytase, when grown in PSM broth, were reduced. Thermophilic moulds exhibited varied levels (0-4651.23 U l\(^{-1}\)) of phytase secretion in PSM broth. Based on higher enzyme production, two different strains of *S. thermophile* (*S. thermophile* BJTLR50, and *S. thermophile* BJA64) and two strains of *Humicola lanuginosa* (*H. lanuginosa* DCC and *H. lanuginosa* BJPAT102) were selected for further screening in order to select a potent phytase producer. Based on repeated screening, *S. thermophile* BJTLR50 that produced higher phytase titre than other fungi, was selected for detailed investigation.

**Phytase Production by *S. thermophile***

**Submerged Fermentation**

Phytase synthesis is inducible in *S. thermophile*, and therefore, glucose-yeast extract-tryptone (GYT) broth with sodium phytate was chosen for enzyme production, as it supported a high enzyme production. Phytase production was enhanced further by optimizing culture variables using Plackett-Burman (P-B) design and response surface methodology (RSM). Phytase production was sustainable in Erlenmeyer flasks of varied volumes and in a fermenter (22 l), suggesting the possibility of scale up of phytase production. To minimize process cost, phytase production was also carried out in a medium containing cane molasses as carbon and energy source. To enhance phytase production, medium components were further optimized by approaches using P-B design and RSM. P-B design analysis suggested that phytase production is affected by (NH\(_4\))\(_2\)SO\(_4\), Tween 80, MgSO\(_4\) and incubation period as indicated by their F-values and p-values. Phytase production got doubled by optimization using statistical methods.

Among different surfactants tested, maximum phytase secretion was observed in a medium containing non-ionic detergents (Tweens) in comparison with control. Tween 80 caused high enzyme secretion as compared to Tween 20 and Tween 40. Triton X-100, a non-ionic detergent, and SDS, an anionic detergent, however, reduced enzyme secretion. Phytase production was highest at water activity (\(a_w\)) 0.95, and thereafter, it declined drastically.

Phytase production by *S. thermophile* was sustainable in 22 l stirred tank and airlift bioreactors. A peak in phytase production was attained in 48 h in fermenters, and there was a slight enhancement in phytase yield in fermenters.

**Solid State Fermentation (SSF)**

Among various agro-residues (wheat bran, wheat straw, corn cob, sugarcane bagasse, and oilseed cakes of cotton, rapeseed and sesame) used, sesame oil seed cake was found to support a high phytase production (148 U g\(^{-1}\)DMR) by *S. thermophile* in SSF. Sequentially statistical-based experimental designs (P-B design & RSM) were applied to further enhance phytase production in SSF. Glucose, ammonium sulphate and incubation period were identified as most significant factors by P-B design, and these were further optimized by RSM.

**Biochemical Characterization of Phytase**

Thermal stability and kinetics of any enzyme are useful in determining its applicability in biotechnological and food industries. Phytase of *S. thermophile* was purified to homogeneity using acetone precipitation followed by ion-exchange and gel-filtration chromatography. Enzyme activity was not inhibited by EDTA, β-mercaptoethanol, dithiothreitol, N-ethylmaleimide, N-bromosuccinimide and phenyl methyl sulfonyl fluoride. Among inhibitors tested, 2,3-butanedione strongly inhibited enzyme activity, suggesting a possible role of arginine residue in catalysis. N-terminal and MALDI-LC-MS/MS peptide
sequences of protein did not show any significant homology with known phytases.

Applications of *S. thermophile* Phytase

Applicability in Dephytinization of Food Ingredients

Phytase dephytinized wheat flour, sesame oilcake and soymilk efficiently with reduction in phytic acid and liberating inorganic phosphate. There was a gradual increase in inorganic phosphate liberation with increase in incubation time with phytase. Dephytinization of food ingredients was higher at 60°C as compared to 37°C, because phytase is optimally active at 60°C.

Role in Bread Making

Dough supplementation with phytase from *S. thermophile* resulted in liberation of higher inorganic phosphate, higher reducing sugars and higher soluble protein than control bread, made with commercial enzymes. Addition of α-amylase and phytase to dough further improved quality and properties of bread as compared to control bread prepared using commercial enzymes. Phytic acid content of wheat flour was also reduced with phytase supplementation.

Plant Growth Promotion

Phytase released inorganic phosphate from calcium, magnesium and cobalt phytates efficiently but it did not hydrolyze Al³⁺, Fe²⁺, Fe³⁺, and Zn²⁺ salts. Among all organic acids, citrate was more effective than malate and oxalate in enhancing phytate hydrolysis by enzyme. Phosphate liberation, reported by Tang *et al*., was lower than that with *S. thermophile* phytase.

Both phytase and mould promote growth of wheat seedlings. When surface-sterilized seeds were germinated on 1/10³ MS agar and seedlings (3-4 cm) were transferred to 1/10³ MS broth (30 ml) containing sodium phytate as P source, both fungus and enzyme promoted growth of wheat seedlings. Growth and inorganic phosphate content of plants were better than control. Effect of different concentrations of sodium phytate was assessed in liquid cultures. Sodium phytate (5 mg plant⁻¹) was adequate for liberating enough P for seedlings growth. Plant growth, root/shoot length and inorganic phosphate content of test plants were better than control.

Compost prepared by combined action of native microflora of wheat straw along with *S. thermophile* promoted growth of plants. Inorganic phosphate content of wheat plants was also high as compared to those cultivated on compost prepared either with only native microflora or *S. thermophile*. Difference in plant growth promoting effect was clear after 10 days, and it further became prominent after 30 days. *S. thermophile* efficiently decomposes plant residues by secreting an array of different enzymes.

Conclusions

Among 138 isolates of thermophilic moulds, *S. thermophiles* secreted high phytase titers. Statistical optimization resulted in improved phytase secretion by mould in submerged as well as solid state fermentations. Phytase production was sustainable in fermenter as well as in Enamel trays, suggesting possibility of scale up of phytase production. Wheat flour, sesame oilcake and soymilk were efficiently dephtylinized by phytase with concomitant reduction in phytic acid content and liberating inorganic phosphate. Addition of phytase to bread reduces phytic acid content and improves bread qualities. Ability to hydrolyze a broad range of organic phosphates including phytates, thermostability, acidic pH optima and insensitivity to trypsin, pepsin and sodium taurocholate make this a good fungal phytase for application in food/feed industries and in plant biotechnology for plant growth promotion. Phytase also finds applications in baking, biopharmaceuticals and combating environmental phosphorus pollution.

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