Prelude & Objectives
Prelude & Objectives

In biological system, phosphomonoester hydrolysis is an important reaction for energy metabolism, metabolic regulation and signal transduction pathways. Phosphorus is an essential element for the growth of all organisms and in livestock production, feed must be supplemented with inorganic phosphorus. **Phytic acid** is the principle storage form of phosphorus, comprising 1-5% by weight in legumes, cereals, oil seeds and nuts, accounting for 60-90% of total phosphorus content in plants where it serves as major storage form of phosphorus, high-energy phosphoryl groups and several divalent cations that can be metabolized by several degradative enzymes during germination. Also, it has been reported to function as natural antioxidant where it acts as potent inhibitor of iron-driven hydroxyl radical formation (Graf et al., 1987). But this phytate phosphorus is largely unavailable to monogastric animals *i.e.*, chickens, swine, poultry animals, humans etc either due to lack of or insufficient amount of phytate degrading enzymes and since phytic acid cannot be resorbed, feeds for pigs and poultry are commonly supplemented with inorganic phosphate. Also the excretion of undigested phytate along with supplementation with inorganic phosphorus imposes global ecological problems (eutrophication) resulting in cyanobacterial blooms, hypoxia and death of marine animals (Reddy et al., 1982). Besides this, phytic acid acts as antinutrient factor, since it forms insoluble complexes with proteins and variety of metal ions, thus decreasing the dietary bioavailability (Wodzinski and Ullah, 1996).

Because of all these problems, there is considerable interest in phytate-degrading enzymes *i.e.*, **Phytases** (*myo*-inositol hexakisphosphate phosphohydrolase). Phytases are histidine acid phosphatases, a subclass of phosphatases, which catalyze the hydrolysis of the phosphate moieties from phytic acid, thereby, resulting in the loss of ability of phytic acid to chelate metal ions. Also the supplementation of animal feed with phytase will increase the bioavailability of phosphorus in monogastric animals besides reducing the phosphorus pollution in the areas of intensive livestock units that result from the excretion of phytic acid and phosphate supplementation (Erdman and Poneros, 1989; Yano et al., 1999; Mallin, 2000; Naqvi et al., 2000). As these phytases catalyze the hydrolysis of phytic acid in a stepwise manner, all utilizing a phosphohistidine intermediate in their phosphoryl transfer reaction (Mitchell et al., 1997), it results in the production of various lower inositol phosphates, *myo*-inositol and inorganic phosphate.
Thus, phytases become potential candidates for the production of special isomers of different lower phosphate esters of myo-inositol, some of which are considered to be pharmacoactive and important intracellular secondary messengers (Greiner and Konietzny, 1996). The most extensively studied positive aspect of myo-inositol phosphate (InsP$_{1,4,5}$ and InsP$_{1,3,4,5}$) is their potential for reducing the risk of colon cancer. The position of phosphate group on inositol ring is, thereby, of great significance for their physiological function (Greiner et al., 2000). To investigate the physiological effects of defined myo-inositol phosphate isomers, these compounds have to be available in pure form and sufficient quantity. Attempts to produce the defined isomers of lower InsPs non-enzymatically resulted in the mixture of isomers (InsP$_5$, InsP$_4$, InsP$_3$, InsP$_2$), thereby, making the purification very uneconomical and arduous task. Thus, a better way to produce these isomers would be to use phytases, which are capable of sequentially hydrolyzing phytate. As the phytases are widely distributed in nature, thus using different type of phytases viz., 3-phytases, 4-phytases and 6-phytases, indicating the predominant attack of susceptible phosphoester bond, may lead to the production of different isomers (Greiner and Konietzny, 1996).

Although a sizeable number of phytase producing organisms are reported, but it has been observed that a thermostable and acid stable phytase with broad substrate specificity and high specific activity is still highly desirable for animal nutrition purposes and is of great commercial importance. Wyss et al., (1999b) studied various reported phytase-producing isolates and showed that the phytases with broader substrate specificity generally had low specific activities. Despite the considerable economic interest, low yield and high cost of enzyme production are the limiting factors in using this enzyme in animal diet. India is presently using dicalcium phosphate (DCP) in animal feeds and it was seen that the phytase supplementation could replace 50-60% dicalcium phosphate. It is estimated that 10 kg DCP can be replaced by 250 gm of phytase enzyme, thus, knowing the fact that 50-60 % DCP can be replaced by phytase, the potential demand for phytase in cattle and poultry feed will be around 4000 tonnes per annum (www.tidco.com). As not all the livestock units depend upon commercial feed, therefore, the demand level is approximately 200 tonnes per annum. Thus to obtain better and alternative source of phytases, there is an on going interest in screening new organisms producing novel and
efficient phytases with the ultimate aim to produce this enzyme to cost effective level and establish the suitability for its industrial application.

From the literature it has been found that not much work is reported on the fermentative production of phytase. To the best of our knowledge no substantial amount of work is being carried out in India on the production and utilization of phytase in feed and fodder industry. Thus, the present work was aimed to develop a process for phytase production with the following objectives:

i) Screening, isolation and identification of a new hyper-producing strain of phytase.

ii) Optimization of medium components and process parameters to maximize phytase productivity in shake flask.

iii) Detailed studies in controlled environment to maximize phytase productivity in laboratory-scale fermenter

iv) Purification and biochemical characterization of phytase.

v) The subsequent utilization of phytase for increasing the digestibility of live stock feed

vi) Identification of various hydrolytic and dephosphorylated products of myo-inositolhexakisphosphate formed by enzymatic hydrolysis.