Enzymes are well-known biocatalysts and their discovery is remarkable in the field of bioprocess technology. Enzymes are biocatalysts that catalyze various biochemical reactions in the living organisms. Apart from being catalytic in vivo, enzymes can also act as catalysts in vitro for various reactions. The enzymes find application in various industries, including food, feed, pharmaceutical, diagnostics, detergent, textile, paper, leather, and fine chemical industries and also have proven utility in bio-conversions and bio-remediation (Nigam, 2013). These bio-industries require enzymes possessing special characteristics for their applications in processing of substrates and raw materials. The special characteristics of enzymes are exploited for their commercial interest and industrial applications, which include: substrate specificity, tolerance and stability to a varied range of pH and temperature and other harsh reaction conditions, high rate of reaction and biodegradability (Adrio et al., 2014). These unique properties of enzymes allow enzyme-assisted processes in industry to run at milder reaction conditions, with improved yields and reduced waste generation (Jegannathan and Nielsen, 2013). Industrial applications represent more than 80% of the global market of enzymes. Industrial production of enzymes requires a clear understanding of the associated scientific and technological issues. These issues range from identification of the biological sources for enzyme production, their genetic
manipulation for overproduction, strategies for cell cultivation, isolation, purification and stabilization (Sarrouh et al., 2012).

Microbial enzymes are known to be superior enzymes obtained from different microorganisms particularly for applications in industries on commercial scales. Though the enzymes were discovered from microorganisms in the 20th century, studies on their isolation, characterization of properties, production on bench-scale to pilot-scale and their application in bio-industry have continuously progressed, and the knowledge has regularly been updated. Many enzymes from microbial sources are already being used in various commercial processes. Selected microorganisms including bacteria, fungi and yeasts have been globally studied for the biosynthesis of economically viable preparations of various enzymes for commercial applications. More than 3,000 different enzymes have been described in which the majority has been isolated from mesophilic organisms. Enzymes for industrial use are produced by growing bacteria and fungi in submerged or solid state fermentation (Pandey et al., 1999). These enzymes mainly function in a narrow range of pH, temperature and ionic strength.

The microbial enzymes act as biocatalysts to perform reactions in bioprocesses in an economical and environmentally-friendly way as opposed to the use of chemical catalysts (Nigam, 2013). Environmental pollution is no longer accepted in technological societies. Over the past century, there has been a tremendous increase in awareness of the effects of pollution, and public pressure has influenced both industry and government. There is increasing demand to replace some traditional chemical processes with biotechnological processes involving microorganisms and enzymes. The role of enzymes in many processes has been known for a long time. With better knowledge and
purification of enzymes the number of applications has increased, and with the availability of engineered enzymes a number of new possibilities for industrial processes have emerged (Beg et al., 2003).

In conventional catalytic reactions using biocatalysts the use of enzymes, either in free or in immobilized forms, is dependent on the specificity of enzyme. In recent advances of biotechnology, according to the requirements of a process, various enzymes have been and are being designed or purposely engineered. Various established classes of enzymes are specific to perform specialized catalytic reactions and have established their uses in selected bioprocesses. A large number of new enzymes have been designed with the input of protein-engineering, biochemical-reaction engineering and metagenomics. Various molecular techniques have also been applied to improve the quality and performance of microbial enzymes for their wider applications in many industries (Chirumamilla et al., 2001). As a result, many added-value products are being synthesized in global market with the use of established bioprocess-technology employing purposely engineered enzymes.

Among the various industrially important enzymes, hydrolases are the most exploited class of microbial enzymes such as amylases, cellulases, xylanases, proteases and lipases etc. Phosphatases (orthophosphoric monoester phosphohydrolases) amongst the hydrolases are one of the most important groups of enzymes used for industrial applications. Phosphatases are a diverse class of enzymes which catalyzes the cleavage of monophosphoester bonds in various organo-phosphate compounds. However, these enzymes are virtually unable to hydrolyze the monophosphoester bonds in phytic acid. Since the hydrolysis of phytic acid is of great importance, a special class of enzymes hydrolyzing phytic acid has evolved - the phytases (Nannipieri et al., 2011).
Phytases (myo-inositol hexakisphosphate phosphohydrolases) are phosphohydrolytic enzymes that catalyze the hydrolysis of phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate) to myo-inositol phosphate derivatives (in some cases to free myo-inositol - an important growth factor) and releases inorganic phosphate in a stepwise manner, all utilizing a phosphohistidine intermediate in their phosphoryl transfer reaction (Vats and Banerjee, 2004).

Cereals, legumes and oilseed crops are grown over 90% of the world’s harvested area. Together they serve as a major source of nutrients for the animal kingdom. An important constituent of these crops is phytic acid. The salt form, phytate, is a major organic storage form of phosphate that represents 1-1.5% of weight and 60-80% of the total phosphorus in seeds (Lei et al., 2013). Phytic acid is also a storage form of myo-inositol. In addition, phytic acid and myo-inositol derivatives derived from it, serve several other important physiological functions in plants, especially in seed germination (Reddy et al., 2001). Due to its chemical structure phytic acid is a very stable molecule. It differs from other organo-phosphate molecules in having a high phosphate content, which results in a high negative charge over a wide pH range. Under normal physiological conditions, phytic acid chelates essential minerals such as calcium, magnesium, iron and zinc (Maga, 1982). Phytic acid also binds to amino acids and proteins and inhibits digestive enzymes (Pallauf and Rimbach, 1996). Thus, phytic acid is an antinutritive component in plant-derived food and feed, and therefore enzymatic hydrolysis of phytic acid is desirable.

The ruminant animals digest phytic acid through the action of phytases produced by the anaerobic gut fungi and bacteria present in their rumenal microflora. However, monogastric animals such as pig, poultry, swine, fish
and human, utilize phytate phosphorus poorly because they are deficient or lack adequate levels of phytate degrading enzymes (phytases) in their gastrointestinal gut. Therefore, supplemental inorganic phosphate is added to their feed to meet the phosphate requirement and to ensure good growth. Rock phosphate soon proved to be a cost-effective means of supplying this additional phosphorus, and the excess phytin phosphorus could be disposed of easily with the animals manure creating massive environmental problems. However, supplemental inorganic phosphate does not diminish the antinutritive effect of phytic acid. The antinutritive effect of phytic acid is especially problematic in the feeding of fish, due to their short gastrointestinal tract (Cao et al., 2007).

The problems created by simple-stomached or monogastric animals could be solved by hydrolysis of phytate using supplemental phytase (Simell et al., 1989). Therefore, phytase has become an important industrial enzyme and is the object of extensive research. By working efficiently on the substrate in the prevailing conditions, supplemental phytase could diminish the antinutritive effects of phytic acid and reduce the cost of diets by removing or reducing the need for supplemental inorganic phosphate. In addition, phytase would be an environmentally friendly product, reducing the amount of phosphorus entering the environment (Wodzinski and Ullah, 1996).

Phytases are now-a-days used commercially in the animal feed industry to improve animal performance and also help in reducing environmental problems caused by monogastrics. The influx of phosphorus in fresh water bodies can lead to eutrophication resulting in algal or cyanobacterial blooms, hypoxia, death of fish and aquatic animals and production of nitrous oxide, a potent greenhouse gas. The projected growth of livestock industry is expected
to accelerate such environmental problems on a global scale. Supplementation of animal feeds with phytase is desirable to reduce the amount of phosphorus excreted (Ciofalo et al., 2003). Another area that offers tremendous opportunity is the use of phytase in aquaculture. Research is currently centered on utilizing phytase to allow producers in this industry to switch to lower-cost plant protein in their fish feed formulations. Other areas for expanded use range from the use of phytase as a soil amendment, to its use in a bioreactor to generate specific myo-inositol phosphate species. The transformation of phytase into a peroxidase may lead to another novel use for this enzyme. In expanding the use of phytase, another important consideration has been achieved. Conservation of the world's deposits of rock phosphate is recognized as important for future generations.

Phosphorus is a basic component of life like nitrogen, but, unlike nitrogen, phosphorus does not have a cycle to constantly replenish its supply. It is very likely that the use of phytase will expand as the need to conserve the world's phosphate reserves increases (Mullaney et al., 2000). During the last few decades, phytases have attracted considerable attention from both scientists and entrepreneurs in the areas of nutrition, environmental protection, and biotechnology. In view of ever increasing demand of phytases, there is ongoing interest in isolating new microbial strains producing efficient phytases.

Phytase is widespread in nature. Besides, microorganisms such as bacteria, yeast and filamentous fungi, phytases also occur in plants, and in some animal tissues (Pandey et al., 2001; Vohra and Satyanarayana, 2003; Vats and Banerjee, 2004). They have been studied most intensively in the seeds of plants (Gibson and Ullah, 1988; Greiner, 2002). However, for
commercial applications, industrial enzymes of microbial origin are preferred due to their multifold properties and easy extraction procedure. The bio-efficacy of microbial phytases has been reported to be 1.5 folds as that of plant phytases (Zimmermann et al., 2002).

Selection of particular microbe depends on the nature of substrate, environmental conditions and desired final product. Techniques using solid-state fermentation (SSF) and submerged fermentation (SmF) have been employed for phytase production. The culture conditions, type of strain, nature of substrate and availability of nutrients should be taken into consideration for selecting a particular production technique, as they are the critical factors affecting the enzyme yield. Thermophilic fungi have complex or unusual nutritional requirements and are well-known to produce phytase (Pandey et al., 2001; Bogar et al., 2003; Chadha et al., 2004; Singh and Satyanarayana, 2006). Most of the research on microbial phytase was reported on fungi belonging to genus Aspergillus and bacteria: Bacillus species and Klebsiella species (Pandey et al., 2001; Vohra and Satyanarayana, 2003). Phytase production from yeasts has been fairly well investigated. Among yeast, extracellular phytase is produced by Schwanniomyces sp. (Segueilha et al., 1992), Pichia sp. (Nakamura et al., 2000), Candida sp. (Quan et al., 2001) etc. while intracellular phytase is also known to be produced by some yeasts (Vohra and Satyanarayana, 2001; Pavlova et al., 2008; Georgiev et al., 2013).

Despite considerable economic interest only limited data on the catalytic properties of about a dozen microbial phytases, including bacteria, fungi and yeast is available. Further active research must, therefore, be directed to identifying new native phytase proteins from diverse microflora and plants that would form the basis for creating consensus phytase using genetic and protein
engineering approaches (Adrio et al., 2014). Although several phytases have been isolated, cloned and characterized the “phytase story” is far from being told. An optimal phytase for industrial applications is still lacking. Therefore, there is a constant need for new phytase candidates.

Immobilized enzymes are currently the subject of considerable attention for their advantages over soluble enzymes and the steadily increasing number of applications for immobilized enzymes. Immobilized enzymes are preferred over their free counterpart due to their prolonged availability or reusability that reduces or minimizes downstream and purification processes. With this view, the properties and applications of immobilized phytases from microorganisms have been studied by many researchers (Dischinger and Ullah, 1992; Liu et al., 1999; Quan et al., 2003; In et al., 2007; Singh and Satyanaryana, 2008; Blackburn et al., 2011).

Phytase research efforts now are focused on the engineering of an improved enzyme. Improved heat tolerance to allow the enzyme to survive the brief period of elevated temperature during the pelletization process is seen as an essential step to lower its cost in animal feed (Vats and Banerjee, 2004). Information from the X-ray crystal structure of phytase is also relevant to improving the pH optimum, substrate specificity, and enzyme stability. Several studies on new strategies that involve synergistic interactions between phytase and other hydrolytic enzymes have shown positive results. Further reduction in the production cost of phytase is also being pursued. Several studies have already investigated the use of various yeast expression systems as an alternative to the current production method for phytase using over expression in filamentous fungi. Expression in plants is underway as a means to commercially produce phytase, as in biofarming in which plants such as
alfalfa are used as "bioreactors," and also by developing plant cultivars that
would produce enough transgenic phytase so that additional supplementation
of their grain or meals is not necessary. Ultimately, transgenic poultry and
hogs may produce their own digestive phytase (Lei et al., 2013).

Enzymes are a very well established product in biotechnological
industries, where sales from US have been from $1.3 billion in 2002 to US
$5.1 billion in 2009 and are anticipated to reach $7 billion by 2013. A recent
survey on world sales of enzymes ascribes 31% for food enzymes, 6% for feed
enzymes and the remaining for technical enzymes (Sarrouh et al., 2012). Since
phytase discovery in 1907, a complex of technological developments has
created a potential $500 million market for phytase as an animal feed additive
and growing at a rate of over 10% per annum. The current inclusion rate of
phytase in all diets for swine and poultry is approximately 70%. The current
commercial feed supplement is a recombinant Aspergillus niger (formerly
called as A. ficuum) phytase ((Wodzinski and Ullah, 1996) has been
introduced with the trade name ‘Natuphos’ in animal feed industries of
Europe, Asia, Canada and United States. The enzyme potentially increases
phosphorus availability in the feed by as much as 30%, allowing producers to
reduce inorganic phosphorus supplementation by up to 17%. The phytases
from other microbial sources such as Peniophora lycii and Escherichia coli
have been recently commercialized as feed additive in animal feed industry for
gaining attention towards fattening of chickens, turkeys, ducks and piglets,
laying hens to increased egg production and in increased body weight in
broilers. For its ideal role as an animal feed additive, efforts are now targeted
towards improving its thermostability and pH optimum (Roopesh et al., 2006).
Objectives of the study:

To extend our present knowledge about phytases, it is essential to produce phytase in a cost effective manner. Therefore, the present study was conducted with the aim to isolate and screen potent phytase producing microbial strains and optimize the fermentative production of phytase. The objectives of work are as follows:

1. Collection of soil samples from various locations
2. Isolation and screening of phytase producing microbes by plate assay procedure
3. Cultivation of phytase positive fungal and yeast isolates for maximum phytase enzyme production
4. Optimization of fermentation conditions to enhance phytase enzyme production
5. Partial purification of phytase enzyme
6. Immobilization of partially purified enzyme using suitable matrices and their biochemical characterization
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