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A Culturing of Fungi for Phytase Production by Solid State from Different Sources

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ABSTRACT

Supplementation with phytase is an effective way to increase the availability of phosphorus in seed- based animal feed. Fifteen different types of themophilic fungi such as Aspergillus fumigatus, Curvalaria, Pencillium Sp, Mycrothecicum, Helimenthosporium, Fusaruim Throderna, Alternaria Spices were majorly found during our study they were classified based on the morphological characterization and staining methods This isolates were isolated from the compost moderf various localities. Among all isolates, Aspergillus sp wasfound to be the best isolate for the phytase production. Three different types of materials(rice bran, Poultry soil, Kudithi) were evaluated as growth substratefor phytase production by Sporotrichum thermophile. Of all the sources tested, rice bransupplemented with diluent containing (g/L); $(NH_4)_2SO_4$; 5.0, KH_2PO_4; 1.0, Yeast extract; 2.0 gavemaximum production (4.16 U/mL/min) when 4% volume of the 250 mL conical flask was used after 96 hrs spore inoculation at 45°C using solid-state fermentation.

Key words: Phytase, Kali, Kudithi.

INTRODUCTION

A phytase (myo-inositol hexakis phosphate phosphohydrolase) is any type of phosphatase enzyme that catalyzes the hydrolysis of phytic acid (myo-inositol hexakisphosphate) — an indigestible, organic form of phosphorus that is found in grains and oil seeds— and releases a usable form of inorganic phosphorus[1]. While phytases had been found to occur in animals, plants, fungi and bacteria, phytases had been most commonly detected and characterized from fungi [2]. In modern age of biotechnology, enzymes have proved their market demand over other products of biotechnology with annual sales close to 2.0 billion dollars. Phytases (EC 3.1.3.8), phosphatases that catalyze the hydrolysis of phosphate moieties, have a big share in enzyme business due to its widespread application as a feed supplement[3- 6]. The phytases enhance the bioavailibility of minerals, protein and phosphorus in monogastric animals. They reduce the phosphorus pollution in areas of intensive livestock production [7, 8]. The thermostability of phytase suggests potential biotechnological applications in the pulp and paper industry as a biological agent to remove plant phyticacid. The enzymatic degradation of phytic acid will not produce toxic by-products, so it is environmental friendly [10]. A large number of microbes including bacteria, yeast and filamentous fungi has been used for phytase production. Selection of particular microbe depends on the nature of substrate, environmental conditions and desired final product. Thermophilic fungi havecomplex or unusual nutritional requirements and well-known microbes to producephytase[11-14]. In view of increasingdemand for phytase it is essential to produce phytase in a cost-effective manner. Phytase required for commercial feed preparation must meet following specifications i.e., thermo stability and activity over wider pH range, which is only possible withthermophilic fungi. Poultry manure is a useful nutrient source supplies phytase and high available phosphorous corn diets on the solubility and plant uptake of P, Cu, and Zn in poultry manure and soils amended with

manure.Therefore, the present study was conducted with the aim to isolate apotent strain of thermophilic fungi from local habitat and optimize after screening for theproduction of phytase.

Aims and objectives

1. To isolates and characterize the fungal organism, which hydrolyses phytase enzyme in high amounts.
2. To estimate the amount of Phytase enzymeproduced from fungal micro-organisms from open natural fermenters and from soil near poultry farms.
3. Optimization of the condition for the activity of the enzyme phytase.

MATERIAL AND METHODS

Source of samples

Soil sample from Poultry farm waste, 2 Kali is a fermented waste water of rice and this is used by villagers to cook cereal food. These liquid feed of cattle are a good source of microbes,contributing to vitamins and enzymes in food. They contain a lot of bran – derived materials including phytase.

Isolation of organisms

Three different samples were used to isolate fungi which have the ability to produce phytase enzyme during the growth cycle. The poultry soil, kali and kudith samples were collected from Warangal and its surrounding areas. Samples were processed by serial dilution method [15]. Soil samples of poultry farms were collected in sterile polythene bags. One gram of sample was suspended in 100 mL of sterilized distilled water and allowed to settle overnight at room temperature. The soil suspension was further diluted up to 104-106 times. One millilitre of this dilute suspension was then transferred to ndividual Petri plates containing potato dextrose agar medium. The fungal cultures were further purified from bacterial contaminants by using 10 mg/L combination of penicillin and streptomycin (1:1 ratio) in the Petri plate medium. Two other samples Kudithi and Kali were collected in sterile bottles from different cattle sheds and from different houses of suburban locatlities of Warangal town.

Morphological appearance

All the isolates of fungi were identified by microscopic examination [16,17,18]. Independent colonies of each identified isolate were picked up and transferred to potato dextrose agar (PDA) slants for culture maintenance. The cultures were stored in a refrigerator at 4°C for further studies.

Inoculum

The spores from 3-5 days old slant culture were wetted by adding 10 mL ofsterilized 0.005% Monoxal O.T (diacetyl ester of Sodium sulphosuccinic acid) to eachslant. The spores were scratched with sterilized inoculating needle and the tubes wereshaken gently to break the clumps of spores. sporesl suspension was used as aninoculum. Inoculum size was measured by measuring the density of spore(number of spore per unit volume) with Haemacytometer, Neubauer improved; precicdor HBG, Germany (Tiefe depth profondeur 0.10 mm and 0.0025mm2 area).

Phytase assay

Phytase activity was assayed after some modification of [12] and [13] methods using Sodium phytate as substrateand the inorganic phosphorus released was measured spectrophotometerically by usingthe Taussky-Schoor reagent. Half milliliter of Sodium phytate (0.00682M) was added to0.1 mL of $MgSO_4$ (0.05M) and 0.1mL of Sodium acetate buffer (0.2M). Enzyme solution(0.1 mL) was added to above mixture and the mixture was incubated at 50°C for 30minutes. After incubation, 1.0 mL of 10% tricarboxylic acid (TCA) was added along with2.0 mL of distilled water. Mixed well and 5.0 mL of Taussky-Schoor reagent was added.Taussky-Schoor reagent was prepared when 10.0 g Ammonium molybdate was mixedwith 10.0 mL H_2SO_4 (10N) and further diluted with 70.0 mL of deionized water. Then 5.0g of ferrous sulphate heptahydrate ($FeSO4.7H_2O$) was added and made the final volumeupto 100.0 mL. Absorbance was measured at 660 nm by using spectrophotometer andliberated inorganic phosphate was estimated after comparing the absorbance with known concentration of KH_2PO_4 using same assay conditions instead of enzyme. One unit ofphytase activity is defined as “the amount of enzyme that liberates one µmol of inorganicphosphate at temperature (50°C) and pH (5)”.

Statistical analysis

Duncan's multiple range tests in the form of probability <p> valueswere used to find out the significant difference among replicates. Treatment effects werecompared after Snedecor & Cochrane (1980) using computer software Costat, 3.03Berkeley, CA 94701.

RESULTS AND DISCUSSION

Both Kudithi and Kali contained microbes which had ability of producing phytase. Fungi Seven strains of five different thermophilic fungi such as Aspergillus fumigatus,Humicola insolens, Rhizomucor miehei-I & II, Sporotrichum thermophile, Thermomyceslanuginosus-I & II were isolated from compost soil and were screened for phytaseproduction. *Aspergillus fumigatus* produced 0.04 U/mL/min of phytase while Humicolainsolens, Rhizomucor miehei-I & II, Sporotrichum thermophile, Thermomyces lanuginosus-I & II gave 0.22, 0.20, 0.76, 2.20, 0.20 and 0.53 U/mL/min respectively. Of the seventhermophilic fungal strains screened for phytase production, Sporotrichum thermophile wasfound to produce higher extracellular phytase when grown on solid state wheat bran (Table1). Chadha et al., (2004) isolated and screened out thermophilic fungi and found ninethermophilic strains having the potential of phytase production like Rhizomucor pusillus,Humicola grisea, Sporotrichum thermophile, Humicola insolens, Thermomyceslanuginosus-I, Thermomyces lanuginosus-II, Rhizomucor miehei-I, Rhizomucor miehei-IIand Aspergillus fumigatus. Singh & Satyanarayana (2008) also investigated thatSporotrichum thermophile has the potential for enhanced production of phytase.The main product of a fermentation process often determines the choice of carbonsource, particularly if the product results from the direct dissimilation of it. It is commonpractice to use carbohydrates as carbon source in microbial fermentation processes. Themost widely

CONCLUSIONS

Phytase which is liberated during the growth cycleof microbes especially fungi and bacteria, thephytase enzyme play a vital role in improving the nourishment of plants which is a rich sourceof carbon source, the study was based on the background of Indianvillage feeds for animals which is rich source Of Proteins and carbohydrates this components are been utilized by the fungi when they grow in kali and kudhithi and release phytase enzyme. This phyatatic kali and kudhithi was been used as feed for animals but there is a complication in digestion of phyatses by animals this is liberated out as indigested produted in to environment. Execrates of animals can be used as manure in fields.

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