ABSTRACT

The present research work is concerned with the cost effective production, purification and characterization of phytase by *Aspergillus niger*. In this context, solid state fermentation (SSF) technique was employed using rice polish as substrate for phytase production. Among various microbial species tested, *Aspergillus niger* was selected as best phytase producer (199 IU/g), whereas, M5 medium was selected as best fermentation medium for better phytase production.

One factor at a time (OFAT) technique was used for the optimization of different cultural conditions and process parameters, and the results showed that glucose 1% (w/w), NH_4NO_3 0.5% (w/w) and tween-40 0.5% (w/w) were found as best carbon source, nitrogen source and surfactant, respectively for improved phytase production (253 IU/g). Whereas, maximum activity of enzyme (297 IU/g) was recorded after 5 days of incubation at 35°C, pH 6, with 80% (v/w) moisture level and 10% (v/w) inoculum size.

The present study was aimed at cost effective and high level production of phytase by *Aspergillus niger* using solid state fermentation. For this purpose, Central composite design (CCD) of Response surface methodology (RSM) was applied for the optimization of significant factors identified previously by Plackett-Burman design (PBD), and incubation period (6 days), incubation temperature (35°C), initial pH (6), NH_4NO_3 (0.75%) and tween-40 (0.6%) were found to be optimum levels for enhanced enzyme production. After statistical optimization, 406 IU/g of phytase production was obtained, as it was 297 IU/g using conventional one factor at a time (OFAT) optimization approach, indicating 1.37-fold increase in phytase yield. These results indicated the efficacy of RSM as optimization technique to enhance the economical production of phytase.

Strain improvement of *Aspergillus niger* was carried out in the presence of ultra violet (UV) irradiation and ethyl methane sulfonate (EMS 0.5% v/v) treatments for various time intervals. We reported an improved strain of *Aspergillus niger* designated as UV-3 mutant which can produce highest phytase (548 IU/g) among all the mutated and wild st[illegible]iving approximately 1.35 fold increase in phytase production compared to v[illegible]ain (406 IU/g) under similar culture conditions. Thus,

physical and chemical mutagenesis was proved to be an efficient technique for strain improvement and ultimately, for enhanced and economical phytase production for different industrial applications.

The crude enzymes produced by wild and selected mutant strains of *Aspergillus niger* were purified through different purification steps i.e. ammonium sulphate precipitation, dialysis and gel filtration chromatography by fast performance liquid chromatography (FPLC) system. This purification step resulted in 39% phytase yield and 13.7 fold purification with a specific activity of 561 U/mg for wild strain phytase compared to mutant strain phytase, which showed 43% phytase yield and 15.2 fold purification and 697 U/mg of specific activity.

For the determination of molecular weight, purified samples of phytase (wild & mutant) were analyzed on 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) by running under denaturing conditions. Both the purified phytase showed a single band after staining with commassie blue R-250. The molecular weight of each phytase band was calculated accordingly and found to be 44.7 kDa.

The results about characterization of phytase revealed that the maximal enzyme activity was found at 50°C, pH 5, substrate (phytic acid) concentration 1% (w/v) incubated for 10 min. It was also observed that Ca and Mg ions had considerable stimulatory effect, whereas K ion showed a slight positive effect, however, the metal ions such as Mn, Na, Fe, Zn and Cu exhibited inhibitory effects on phytase activity. Thermo-stability and pH stability of the purified enzyme was also studied. The results showed that enzyme was stable at low temperatures (50°C) and at pH 5.

At the end, phytase enzyme was used as poultry feed additive to analyze its effect on the growth of chicks. The results indicated the positive effect of phytase on chicks growth and body weight gain (BWG).