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Production and purification of phytase by solid state fermentation from Rhizopus oligosporus MTCC 556 using agricultural wastes

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Phytic acid (myo-inositol 1,6 hexakisphosphate) is the major storage form of phosphorus in plants. It cannot be metabolized in monogastric animals since phytic acid acts as an anti-nutritional agent by chelating several metal ions and insoluble form of protein complex. This consequence resulted in mineral deficiencies and phosphorus pollution. One of the finest ways to utilize the phosphorus from phytate is the use of phytase enzyme. In this study, Rhizopus oligosporus MTCC 556 was used to produce phytase by solid state fermentation (SSF) using six different agricultural residues as substrates. A maximal enzyme production of 15.8 U/gds was observed with wheat bran as substrate. Three-step purification was employed with ammonium sulfate precipitation, ion exchange and gel filtration chromatography which resulted in 14.6-fold purified phytase. The molecular weight was estimated to be 88Kda by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The optimum pH and temperature for phytase activity was found to be 5.5 and 55°C respectively. The effect of different metal ions was examined which showed that Mg^{2+} and Zn^{2+} ions enhanced the phytase production significantly. This study would be useful for reducing the phosphorus pollution and increasing interest towards efficient utilization of agro industrial wastes for enzyme production.

Biography

Suresh S is pursuing his PhD in the department of chemical engineering, Anna University, Chennai. He has completed M.Sc (Biochemistry) during 2007-09 at SRM Arts and Science College, Chennai and also having a teaching experience for one year at Dr. MGR.Chockalingam Arts College, Arni, Thiruvanamalai District, Tamilnadu.

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Production and characterization of monoclonal antibodies against nucleoprotein of avian influenza

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The present study was carried out with the overall objective of developing anti-NP mAbs which can be used in immunochromatographic test based assay and competitive ELISA (C-ELISA) for the rapid detection of avian influenza virus (AIV) antigen and antibodies, (Abs) respectively. The recombinant NP antigen (rNP Ag) was expressed in E. coli BL 21 strain as approx 57^{+} kDa protein mainly as soluble fraction. Immunoblotting with anti-His HRPO conjugate revealed strong reactivity with the recombinant protein confirming the expression of 6X his-tagged protein in E. coli BL21 strain. The recombinant NP was used to immunize Balb/c mice to produce hybridomas secreting anti-NP mAbs. Seven anti-NP hybridoma clones could be recovered with OD in ELISA ranging from 0.223 to 1.378. After subcloning, total of 9 mAbs were recovered and were further characterized. Out of 9 mAbs, 8D2-H5, 8D2-H9 and 6D11-A7 were of IgM isotype, 5D10-C9 and 5D10-F11 were of IgG2b type while 3F3-D2, 7D2-G7, 7D2-G8 and 7D2-C9 were of IgG1 isotype. The mAbs 3F3 and 7D2 showed high intensity positive reaction with rNP and a low intensity reaction with H5N1 virus in western blot analysis. The anti-NP mAbs produced in the present work may be valuable in developing a Competitive ELISA or Immunochromatographic strip test based assays for the rapid diagnosis of avian influenza.

Biography

Sushant Bhat completed his M.V.Sc in Veterinary Immunology at the age of 25 years from Indian Veterinary Research Institute (deemed university). He completed his research work in High Security Animal Disease Laboratory (OIE reference laboratory for Avian Influenza), Anand Nagar, Bhopal. Presently he is pursuing his PhD in Veterinary Immunology from Indian Veterinary Research Institute, Izatnagar, Bareilly, UP, India.

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