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Studies on L-asparaginase from *Fusarium culmorum* for commercial exploitation

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Three hundred and sixty soil fungi were screened for L-asparaginase production by rapid screening method. *Fusarium culmorum* showed appreciable amount of enzyme activity. Optimization studies under Submerged Fermentation (SmF) revealed that the production of enzyme was maximum at day 4, pH 7.5, temperature 30°C and at 1% substrate concentration. Addition of 0.2% citric acid, 0.5% ammonium chloride, 0.002% calcium chloride enhanced the production by 6 fold. L-asparaginase was purified by ammonium sulphate precipitation, gel filtration and ion exchange chromatography. Fourteen fold increase with specific activity of 16.66 U/mg proteins with 2.6% yield. The molecular weight of the enzyme was estimated to be 90 kDa. The optimal pH and temperature of purified enzyme was 8.0 and 40°C. The enzyme retained 90% activity at pH 8 after 72 hrs and 50% activity at 60°C for 60 min. The K_m and V_{max} of purified enzyme was 3.57 mM and 0.5µmol/ml/min at 37°C respectively, activated by Mn^{2+} and Tween 80, inhibited by Cu^{2+} and EDTA. Production of L-asparaginase was also carried out under Solid State Fermentation (SSF). Sixty five substrates were used; soya bean meal enhanced the production by 10 fold. Soya bean meal in combination with wheat bran and 0.1% ammonium chloride further enhanced the production by 14 fold. The purified L-asparaginase showed cytotoxic effect on human leukemic cell line (Jurkat) with IC_{50} value of 90 µg/ml. The enzyme did not elicit any immunogenic effects on human lymphocytes. The enzyme induced apoptotic cell death by arresting the growth of cells at G2-M phase.

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Proteomic analysis of *Arabidopsis thaliana* (L) Heynh in response to *Plutella xylostella* (L) infestation

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The interaction of *Arabidopsis thaliana-Plutella xylostella* has been considered as a model system to unravel the responsive resistance of plants to herbivorous insects. In the present study, a proteome study using 2-DE was undertaken to investigate the alterations of global proteins in leaves of Arabidopsis plants exposed to *P. xylostella* larval infestation at 27° Cover 0-8 hours. Approximately 454 protein spots were reproducibly detected on each gel by progenesis same spots software. Statistical evaluation of relative spot volumes showed that 18 protein spots differentially expressed in un-infested and infested plant leaves. By using 2-DE coupled with MALDI-TOF MS and LC-ESI-MS/MS, 14 out of 18 differentially expressed protein spots were successfully identified. The results showed that 16 proteins contained in these spots participate in different physiological processes. Of these, we identified 10 proteins by MALDI-TOF MS and 6 proteins by LC-ESI-MS/MS. The results indicated that 12.5% of the analyzed spots contain multiple proteins. Functional classification analysis indicated that the differentially identified proteins were associated with amino acid-5, carbohydrate-2, energy-3, lipid-2 metabolism and photosynthesis-4. In addition, their relative abundance was up-regulated or down-regulated according to larval feeding on *Arabidopsis* leaves. The data from this study ascertain the response of *Arabidopsis* to chewing insect infestation and provide valuable new insights for further works in plant-environmental stress interaction.

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