

## Optimization of Fermentation Conditions for Extracellular Production of the Oncolytic Enzyme, L-Asparaginase, by New Subsp. *Streptomyces Rochei* Subsp. *Chromatogenes* NEAE-K Using Response Surface Methodology under Solid State Fermentation

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**Abstract :** L-asparaginase is an important enzyme as therapeutic agents used in combination therapy with other drugs in the treatment of acute lymphoblastic leukemia in children. L-asparaginase producing actinomycete strain, NEAE-K, was isolated from soil sample and identified on the basis of morphological, cultural, physiological and biochemical properties, together with 16S rDNA sequence as new subsp. *Streptomyces rochei* subsp. *chromatogenes* NEAE-K and sequencing product (1532 bp) was deposited in the GenBank database under accession number KJ200343. The study was conducted to screen parameters affecting the production of L-asparaginase by *Streptomyces rochei* subsp. *chromatogenes* NEAE-K on solid state fermentation using Plackett-Burman experimental design. Sixteen different independent variables including incubation time, moisture content, inoculum size, temperature, pH, soybean meal+ wheat bran, dextrose, fructose, L-asparagine, yeast extract, KNO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub>, MgSO<sub>4</sub>.7H<sub>2</sub>O, NaCl, FeSO<sub>4</sub>. 7H<sub>2</sub>O, CaCl<sub>2</sub>, and three dummy variables were screened in Plackett-Burman experimental design of 20 trials. The most significant independent variables affecting enzyme production (dextrose, L-asparagine and K<sub>2</sub>HPO<sub>4</sub>) were further optimized by the central composite design. As a result, a medium of the following formula is the optimum for producing an extracellular L-asparaginase by *Streptomyces rochei* subsp. *chromatogenes* NEAE-K from solid state fermentation: g/L (soybean meal+ wheat bran 15, dextrose 3, fructose 4, L-asparagine 8, yeast extract 2, KNO<sub>3</sub> 1, K<sub>2</sub>HPO<sub>4</sub> 2, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.5, NaCl 0.1, FeSO<sub>4</sub>. 7H<sub>2</sub>O 0.02, CaCl<sub>2</sub> 0.01), incubation time 7 days, moisture content 50%, inoculum size 3 mL, temperature 30°C, pH 8.5.

**Keywords :** *streptomyces rochei* subsp. *chromatogenes* neae-k, 16s rrna, identification, solid state fermentation, l-asparaginase production, plackett-burman design, central composite design

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