

## **Extracellular Production of the Oncolytic Enzyme, Glutaminase Free L-Asparaginase, from Newly Isolated Streptomyces Olivaceus NEAE-119: Optimization of Culture Conditions Using Response Surface Methodology**

**Authors :** Noura El-Ahmady El-Naggar

**Abstract :** Among the antitumour drugs, bacterial enzyme L-asparaginase has been employed as the most effective chemotherapeutic agent in pediatric oncology especially for acute lymphoblastic leukemia. Glutaminase free L-asparaginase producing actinomycetes were isolated from soil samples collected from Egypt. Among them, a potential culture, strain NEAE-119, was selected and identified on the basis of morphological, cultural, physiological and biochemical properties, together with 16S rDNA sequence as Streptomyces olivaceus NEAE-119 and sequencing product(1509 bp) was deposited in the GenBank database under accession number KJ200342. The optimization of different process parameters for L-asparaginase production by Streptomyces olivaceus NEAE-119 using Plackett-Burman experimental design and response surface methodology was carried out. Fifteen nutritional variables (temperature, pH, incubation time, inoculum size, inoculum age, agitation speed, dextrose, starch, L-asparagine, KNO<sub>3</sub>, yeast extract, K<sub>2</sub>HPO<sub>4</sub>, MgSO<sub>4</sub>.7H<sub>2</sub>O, NaCl and FeSO<sub>4</sub>. 7H<sub>2</sub>O) were screened using Plackett-Burman experimental design. The most positive significant independent variables affecting enzyme production (temperature, inoculum age and agitation speed) were further optimized by the central composite face-centered design -response surface methodology. As a result, a medium of the following formula is the optimum for producing an extracellular L-asparaginase in the culture filtrate of Streptomyces olivaceus NEAE-119: Dextrose 3g, starch 20g, L-asparagine 10g, KNO<sub>3</sub> 1g, K<sub>2</sub>HPO<sub>4</sub> 1g, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.1g, NaCl 0.1g, pH 7, temperature 37°C, agitation speed 200 rpm/min, inoculum size 4%, v/v, inoculum age 72 h and fermentation period 5 days.

**Keywords :** Streptomyces olivaceus NEAE-119, glutaminase free L-asparaginase, production, Plackett-Burman design, central composite face-centered design, 16S rRNA, scanning electron microscope

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