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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

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Abstract : Among the antitumour drugs, bacterial enzyme L-asparaginase has been employed as the most effective chemotherapeutic agent in pediatric oncotherapy 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, KNO3, yeast extract, K2HPO4, MgSO4.7H2O, NaCl and FeSO4. 7H2O) 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, KNO3 1g, K2HPO4 1g, MgSO4.7H2O 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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