عنوان مقاله:

Optimization of Chemically Defined Cell Culture Media for Recombinant ONTAK Immunotoxin Production

محل انتشار:

فصلنامه میکروب شناسی پزشکی ایران, دوره 8, شماره 3 (سال: 1393)

تعداد صفحات اصل مقاله: 8

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خلاصه مقاله:

Background and Aim: Immunotoxin is a cytotoxic protein and have been proposed as a novel compound for cancer therapy. ONTAK is a recombinant immunotoxin composed diphtheria toxin fused to interleukin 2(IL2). In this study, optimization of defined culture medium for the expression of recombinant ONTAK immunotoxin has been investigated.Materials and Methods: In this study, the bacterial strain BL21 (DE3) was transformed with the recombinant plasmid PET-IDZ was used to express ONTAK. The medium composition such as carbon source (glucose, sucrose and glycerol), nitrogen source (ammonium chloride, urea and ammonium sulfate) and concentration of the inductor IPTG (0.1, 0.5, 1mM), induction time and concentration of various additives (different amino acids) were optimized based on Taguchi method for increasing expression on the shake flask cultivation. The cell concentration was measured by optical density at 600 nm and protein expression levels were analyzed by electrophoresis on SDS-PAGE gel.Results: Modified defined culture medium containing (g/l): glucose,8.0; K2HPO4,15.0; KH2PO4,7.5; Citric acid,2; NH4Cl,3; MgSO4.7H2O,1; were determined as optimal culture medium. OD600nM=2.0 was determined as the best time for induction by IPTG at a concentration of 0.1mM. ONTAK expression was increased by adding Valine, 0.0502; Phenylalanine, 0.0132; Lysine, 0.0184; Aspartic acid, 0.0160 and Serine, 0.0251(g/l) amino acids to the medium. Conclusions: In present study, the Taguchi method analysis revealed that, nitrogen source type (NH4CL 3 g/l) significantly affects in the cell growth. The biomass production was on the optimized medium about over 3 times higher than that at M9 medium.

کلمات کلیدی:

Recombinant Immunotoxin, ONTAK, Optimization