Isolation and identification of bacterial isolates producing Arginine deiminase from assorted soil environments in Egypt using 16S rRNA sequencing technique

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Abstract

Background: Auxotrophic cancers for Arginine are a leading cause of death worldwide. The manufacture of novel arginine degrading enzymes such as Arginine deiminase enzyme is mandatory due to this crisis. Aim of the study: Since certain tumor cells are auxotrophic for Arginine, the depletion of the extracellular Arginine by means of Arginine deiminase enzyme was exploited in the present study to target such tumors. Methodology: Selective recovery of some bacterial isolates from different environmental sites in Egypt and assessment their capabilities for Arginine deiminase production. Studying environmental and physiological factors affecting Arginine deiminase production by some selected isolates. Characterization of activity Arginine deiminase produced by certain selected isolates as well as its production through bacterial recombinant DNA technology.

Results: The major bacterial isolates grown on mineral Arginine agar (MAA) plates producing ADI were further identified as Bacillus subtilis DE 111 using 16S rRNA sequencing technique. The Arginine deiminase production and activity were optimal at 40 and alkaline pH. Mn²⁺, Ni²⁺ and Co⁺² metal ions as cofactors were optimum activators for production and activity of ADI. The results showed that the potent cytotoxic consequences of ADI were exerted on the renal and leukemic cancer cell lines, ADI produced via bacterial recombinant DNA technology showed efficacious IC₅₀ 10.31 ± 0.2 and 16.08 ± 0.3 μγ/μl against renal (Caki-1) and leukemic (K-562) cancer cell lines, respectively. The purified monomeric ADI was 36.18 KDa molecular mass as determined using SDS-PAGE, the specific activity reached 36.07 U/mg. Km, Vmax and Kcat were 0.0587 M, 40.36 μμολ/μλ/μιν and 5.014μιν⁻¹ respectively. Optimum pH and temperature for productivity and activity ranged from 6-10 and 37-70 respectively. Total protein estimation using Bar-ford assay was determined to be 5.68 mg during the initial culture. ADI purification was achieved using 70% Ammonium Sulfate followed by Ni²⁺ immobilized affinity column chromatography with a final purification fold of 15.03. In vitro determination of biological half life of ADI using nesslerization assay was observed to be nearly 300 min. Conclusion: ADI produced from Bacillus subtilis DE111 demonstrated efficacious anticaner activities against leukemic (K-562) and renal (Caki-1) auxotrophic cancers for Arginine due to the depletion of L-arginine from the external surrounding environments.

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