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Identification and characterization of *Bacillus thuringiensis* strains with nematicidalactivity

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Crystal proteins from the soil bacterium B*acillus thuringiensis* (Bt) are globally used in agriculture as biological control agents against insect pest, but its use as a nematicidal control agent is still under development. In this work, a total of 310 Bt strains were screened for activity against the free-living nematode Caenorhabditiselegans. Strains LBIT-596 (serotype darmstadiensis) and LBIT-107 (serotype neoleonensis) showed significant toxicity levels. These strains were characterized by plasmid and RepPCR patterns, and flagellin gene sequencing. Preliminary bioassays of LBIT-596 and LBIT-107 spore-crystal complexes estimated LC50s at 63.36 and 76.33 µg/ml, respectively, and 24.2 and 24.99 µg/ml, respectively, when pure crystals were tested. SDS-PAGE protein content analyses of LBIT-596 crystals showed two proteins (35 and 130 kDa) before activation, which turned into lower molecular-weight proteins (28 and 55 kDa) after activation. LBIT-107 also showed two major proteins of 28 and 70 kDa, before activation. Amplicons from the cry-gene conserved blocks and from cyt1 gene group were cloned and sequenced. Sequence analyses indicated that LBIT-596 contains sequences identified within the cry5B and cyt1A gene families, while LBIT-107 contains sequences identified within the cry14 and cyt1A gene families. Interestingly one of the amplicons from LBIT-107 showed only 88% identity with the cry14A gene. These results indicate a potential use of these toxins against economically important parasitic nematodes.

Recombinant expression, purification of L-asparaginase-II from the rmotolrant *E. coli* strain and evaluation of its antiproliferative activity

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Repeated use of L-asparaginase II enzyme, in the treatment of acute lymphoblastic leukaemia, is commonly needed because of the enzyme's instability and relatively short half-life which leads to more serious side effects on patients. In the present study, we report on the cloning and expression of L-asparaginase from a thermotolerant strain of E. coli (KH027) which isolated from camel manure and can grow at 45° C. Expression of recombinant asparaginase was conducted by fusion asparaginase gene to pelB leader sequence and 6His residues at the C-terminus under the inducible T7 promoter in DH5α cells. Induction of the cells with 0.1mM isopropyl-β-D-thiogalactopyranoside (IPTG) at late log phase of growth resulted in 1.6-fold (2111 UI) higher to that obtained in early log phase induction (1319 UI) and 1.3-fold compared with mid log phase induction (1623 UI). The recombinant asparaginase protein was purified from the culture supernatant through nickel affinity chromatography. The apparent molecular weight of the tetramer enzyme was found to be ~141 kDa. Overall yield (87 mg/L) of the purified recombinant asparaginase was achieved at the shake flask level. The purified protein showed optimum activities at a temperature of 43°C and pH 6. The Km and Kcat parameters were 3.8mM-1 and 2.92 ×103s−1, respectively. The enzyme retained around 57% and 30% of its initial activity after 30 and 60 minutes of incubation at 50 °C, respectively. Recombinant L-asparaginase was evaluated for its antiproliferative effect in the leukemia cell lines of RS4; 11 and HL60 after 96 and 72 h of incubation. The doses of 100µg/mL and time-response effect of 96 h caused a reduction value of 50% in cell viability of RS4. However, cell viability of 50% in the leukemic cells HL-60 was noticed with a concentration of 200µg/mL with an incubation period of 72 h. *In vitro* antiproliferative results in the leukemia cell lines encourage for making in vivo investigation to increase the possibility of using this thermostable enzyme in leukaemia therapy

Biography

Muharram M M has completed his PhD in molecular biology at the age of 35 years from Al-Azhar University, college of science through the channel system with Hamburg University, Germany. His PhD work focused on the characterization of mitochondrial signal peptidases of S. cerevisiae. He has published more than 15 papers in reputed journals. His research interest is in the characterization of therapeutic enzymes and their expression and purification.

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