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Purification of recombinant *Erwinia carotovora subsp. atroseptica L*-Asparaginase II produced in *Escherichia coli*

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Abstract

The bacterial enzyme asparaginase has been used in acute lymphoblastic leukemia (ALL) treatment for approximately 30 years (Amylon et al, 1999; Avramis and Panosyan, 2005; Ortega et al, 1977). Asparaginase hydrolyzes the amino acid asparagine to aspartic acid and ammonia (Kiriyama et al, 1989; Moola et al, 1994; Oettgen et al, 1967; Richards and Kilberg, 2006), cutting the asparagine supply in the blood; as a consequence, the cancer cells die as they become unable to build their proteins (Müller and Boos, 1998). Presently, the asparaginase available for clinical use is isolated exclusively from *Escherichia coli*, *Erwinia chrysanthemi*, and *Erwinia carotovora* strains. The protein obtained from these sources has identical mechanisms of action differing in their pharmacokinetic properties. Nevertheless, asparaginases have limited use as drug because of toxicity, which may cause liver dysfunction, pancreatitis, diabetes, leucopenia, neurological seizures, and coagulation abnormalities that may lead to intracranial thrombosis or haemorrhage (Duval et al, 2002; Oettgen et al, 1970). Another limiting factor of *E. coli* asparaginase is the development of hypersensitivity, which ranges from mild allergic reactions to anaphylactic shock (Moola et al, 1994). The *E. carotovora* asparaginase exhibits fewer side effects when used in the anti-cancer therapy (Krasotkina et al, 2004), being an useful option for patients who develop hypersensitivity to the *E. coli* enzyme (Duval et al, 2002).

Among its array of applications, asparaginase is also used to treat Hodgkin's disease, acute myelocytic leukemia, lymphosarcoma, reticulosarcoma and melanosarcoma (Duval et al, 2002). Interestingly, no reports are available concerning the production of this important anti-cancer drug using recombinant DNA techniques. Commercially available asparaginase is

produced by wild-type gene fermentation but production of large quantities is restricted due to the extremely low efficiency of this process. As a consequence, asparaginase for most clinical applications in Brazil and elsewhere is largely dependent on imports. Needless to say, the extremely high price of this drug represents a key restriction to its clinical use (Guo et al, 2000; Kozac, Jaskólski and Röhm, 2000).

Therefore, this work represents an important step in the production of asparaginase in large quantities for use as an anti-cancer agent, minimizing the bacterial-asparaginase side effects and reducing the costs of enzyme production. For this, the objectives of this work are (a) cloning of *E. carotovora subsp. atroseptica L*-asparaginase II *ErA* gene, (b) protein expression in *E. coli* cells, (c) purification of the recombinant enzyme and (d) measurement of asparaginase activity and kinectic characterization of this enzyme.

E. carotovora asparaginase gene (*ErA*), including the periplamatic signal peptide, was amplified by PCR using a specific primer containing *Nde*I and *Bam*HI restriction sites. Its amplicon was cloned into pCR-Blunt® vector (Invitrogen) and subcloned into pET-30a(+) expression vector (Novagen) which was used to express the recombinant protein in *E. coli* cells. The asparaginase recombinant protein was expressed in the soluble fraction of C41(DE3) strain, and best expression conditions were obtained at 37ºC after 6 hours of incubation with IPTG 1mM induction. SDS-PAGE Analyses were made to confirm asparaginase expression in the periplasmic space and in the culture media. A purification protocol for this enzyme was established on the use of two chromatography steps, a cation exchange (HiPrep SP XL) and a size exclusion (Sephacryl S200) columns. Enzyme assays are currently underway to determine the biological activity and the kinetic parameters of this enzyme.

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