Expression patterns of recombinant asparaginase in different *E. coli* strains

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Abstract

L-Asparaginase enzymes (L-Asparagine amidohydrolase; EC 3.5.1.1) catalyze the hydrolysis of L-Asparagine (L-Asn) to L-Aspartate (L-Asp) and ammonia (NH₃), and to a lesser extent the hydrolysis of L-Glutamine (L-Gln) to L-Glutamate (L-Glu) (Wang et al., 2001). Two types of bacterial L-asparaginases have been identified: type I and type II (Campbell et al., 1967). Type II L-asparaginases, in particular, present tumor inhibitory activity and have thus been extensively studied (Cedar and Schwartz, 1968).

Tumor-inhibitory L-asparaginases have been isolated from a number of bacterial sources. However, their glutaminase activity has been implicated in causing serious side effects. Fortunately, L-asparaginase II from *Erwinia carotovora*, which was used in this work, has significantly lower glutaminase activity and may represent an important alternative therapy (Hawkins et al., 2004; Krasotkina et al., 2004).

The study of the expression patterns of recombinant asparaginase in different *E. coli* strains, which is the aim of this work, is an important step for later commercial production. Among the alternatives for the production of this enzyme, we can name the direct isolation from the microorganism, or the production of the enzyme through recombinant technology in genetically modified *E. coli*. Nowadays, *E. coli* is the most well-studied microorganism. This knowledge allows some easiness in the manipulation of the bacteria and the production of heterologous proteins (Lima, 2004). Genetic manipulation allows the creation of mutant strains with specific characteristics; this differentiation is favorable for better recombinant protein expression.
After amplification of the asparaginase gene from *Erwinia carotovora*, the gene was cloned into the expression vector pET30a(+) and used to transform different *E. coli* strain by electroporation. As control the plasmid lacking the asparaginase gene was used. The *E. coli* strains used were: BL21 (DE3) NH, BL21 (DE3) Star, C41 (DE3), C43 (DE3), Rosetta (DE3), and BL21 (DE3).

The TB culture medium was used at 37°C and 30°C, and was inoculated to an OD\(_{600nm}\) of 0.1. The optical density was monitored at each hour. Cultures were induced, or not, with 1 mM IPTG after reaching OD\(_{600nm}\) 0.4 - 0.6. For control the *E. coli* strain was transformed with the plasmid lacking the asparaginase gene. For expression analysis 1 mL of the cultures were submitted to electrophoresis on polyacrylamide gels (SDS-PAGE). Samples were collected at 3, 6, 9 and 24 hours after induction. In order to separate the soluble and insoluble fractions, the samples were centrifuged at 13,000 rpm for 3 min and frozen at -20°C.

All *E. coli* strains tested showed the superexpression of the asparaginase at 37°C. At the moment, the cultures are being tested at 30°C in order to observe if the temperature will influence in the expression of the protein.

These studies are important for the scaling-up process in the bioreactor, aiming the national production of this enzyme.

**Referências**


