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Studies on industrially and pharmaceutically important L-asparaginase: A prospective biotherapeutic product against leukemia

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L-asparaginase is a pharmaceutically and industrially important enzyme as it has the potential to treat acute lymphoblastic leukemia (ALL) and inhibit acrylamide formation in fried and baked food products. In the present study, three new potential L-asparaginase producing bacteria were screened and identified. During the screening, we have developed a new pH indicator dye-based (cresol red-based) plate assay for the screening of L-asparaginase producers. A cresol red-based method gave a better differentiable zone of hydrolysis and consistent results and helped to avoid problems such as a less differentiable and inconsistent zone of hydrolysis which have reported previously in a phenol red and a BTB-based plate assay.

Further, we performed L-asparaginase bioprocess optimization (conventional and statistical optimization) studies. The investigations resulted in an enzyme production of 37.63 IU/ml from a newly isolated microbe, Pseudomonas resinovorans IGS-131, which was 3.45 fold higher than the initial or un-optimized enzyme activity (i.e., 10.91 IU/ml). This was followed by isolation, cloning, and expression of L-asparaginase gene from Pseudomonas resinovorans IGS-131 in Escherichia coli rosetta DE3, and the gene sequence obtained in this study showed 78% identity with a reported sequence in GenBank. The recombinant L-asparaginase was purified by affinity chromatography and gel filtration methods (size exclusion) to homogeneity with a final protein yield of 62.53 mg/L. The molecular weight of the recombinant protein was determined as 34.7 kDa by SDS-PAGE. The oligomeric state of the protein was determined as around 95% of protein species present as a monomer of 34.7 kDa by analytical ultracentrifugation (AUC). Also, purified protein showed the good antioxidant activity of 62% against free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The current method of screening for L-asparaginases is more rapid and efficient. Also, the recombinant production of this enzyme will help in avoiding the complexity of downstream processing associated with conventional methods.

The L-asparagine enzymatic hydrolysis in L-aspartate and ammonium was observed in a first time by Lang (1904) that detected L-asparaginase activity in bovine's tissues. Results of this researcher were confirmed by Furth and Friedmann (1910) that detected L-asparagine hydrolase in horse and pig organs, observing the same amount of L-asparaginase activity in both animals. Also, Clementi (1922) related that L-asparaginase in guinea pig serum, although antitumor activity of the enzyme was identified only some years later. In addition, Mashburn and Wriston (1964) demonstrated that L-asparaginase of *E. coli* had inhibitory capacity of tumors in rats. However, the large interest in enzyme started when Broome (1965) found that the regression lymphosarcoma transplants in rats treated with guinea pig serum was due to nutritional dependence on malignant cells of exogenous L-asparagine.

Considering its properties, L-asparaginase has been an important chemotherapeutic agent used for treatment of lymphoproliferative and lymphoma diseases. Particularly, it presents large importance in chemotherapeutic protocols for acute lymphoblastic leukemia (ALL) and Hodgkin's lymphomas.

Cancer cells, mainly lymphatic cells, require high amount of asparagine for fast and malignant growth. In this way, cancer cells requires the amino acid from diet (blood serum) as well as amino acids produced by themselves. However, leukemic lymphoblasts and some others tumor cells do not have or present low quantity of L-asparagine synthetase used for L-asparagine syntheses. Thus, these malignant cells are dependent of asparagine from blood serum for their proliferation and survival.

L-asparaginase hydrolyzes asparagine from blood serum, leading tumor cells to death by lacking of an essential factor for protein synthetases (p53-dependent apoptosis). However, healthy cells are not affected, because they are able to produce asparagine using L-asparagine synthetase present in enough quantities. Considering these concepts.

Acrylamide (C3H5NO) is also known as 2-propenamide, acrylic amide, ethylene carboxamide, propenamide, propanoic acid amide, monomer of acrylamide or acrylic acid amide, presenting 71.08 g/mol of molecular mass. Several studies show that L-asparagine is the main amino acid responsible for acrylamide production in fried and baked foods when reducing sugars are condensed with a carbonyl source. This phenomenon does not occur in boiled food.

Acrylamide formation has been quite studied in the last years. Zyzak et al. (2003) detected that the amide chain present in the acrylamide structure is provided from L-asparagine. Reagents (L-asparagine or reducing sugars) reduction or removal is one of the evaluated strategies for decreasing acrylamide quantity in foods. For L-asparagine reduction, several options have been investigated, such as: selection of vegetal species with lower level of L-asparagine in their composition; deletion of important enzymes for L-asparagine biosynthesis control by suppression of specific genes; acid hydrolysis of L-asparagine leading the formation of aspartic acid and ammonia; and acetylation process of L-asparagine to form N-acetyl-L-

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asparagine, preventing the formation of acrylamide from intermediate N-glycosides.

In the study of Zyzak et al. (2003), authors confirmed that the use of L-asparaginase enzyme before frying or baking food process could reduce more than 99% acrylamide level in the processed final product. This is because the enzyme reduces more than 88% of the L-asparagine concentration from the initial feedstock. In last years, other works have dealt with this application of L-asparaginase, that can decrease the negative effects of acrylamide containing foods without impair their characteristics.

L-asparaginase is present in mammals, birds, plants, yeast, and a wide range of bacteria. Although L-asparaginase production is observed in animals, plants, the microorganisms are considered mainly source for L-asparagine synthesis.

The production of this enzyme is mainly proceeded by submerged fermentation. Several researchers have studied the isolation of microbial strains that produce this important enzyme, such as Pseudomonas fluorescens, Serratia marcescens, Escherichia coli, Erwinia carotovora, Proteus vulgaris, Saccharomyces cerevisiae, Karnatakensis Streptomyces, Streptomyces venezuelae and several genres of fungi as Aspergillus, Penicillium and Fusarium.

Concerning to bacteria, the best producers of L-asparaginase are members of the Enterobacteriaceae family. For example, in pharmaceutical industry, this enzyme is produced mainly from bacteria such as Escherichia coli and Erwinia carotova, (also known as Erwinia chrysanthemi), generally used for leukemia and lymphoma treatment. However, most of these treatments can result in immunological sensitization (hypersensitivity) and immune inactivation in patients that receive bacterial enzymes.
Another issue is that glutaminase activity generated by these enzymes can cause secondary effects such as allergic reaction, nausea, pancreatitis, diabetes and coagulation abnormalities. Also, most of asparaginases has low stability and catalytic activity, presenting only active in a narrow pH range.