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Design, construction and extracellular expression of L-asparaginase from *Dickeya chrysanthemi* in yeast

Brian Effer[1,2], **Jorge Farias**[1] and **Gisele Monteiro**[2]
[1]University of La Frontera, Chile
[2]University of São Paulo, Brazil

L-asparaginase (L-ASNase) is an important enzyme used as a biopharmaceutical to treat acute lymphoblastic leukemia (ALL). Currently there are two L-ASNase approved by FDA: native and of bacterial origin, both from *E. coli* and *D. chrysanthemi*. Due to L-ASNase's immunogenic effects, it is necessary to seek alternatives such as recombinant expression in yeast. This expression system can provide extracelullar secretion and glycosilation process, which can decrease immunogenicity and facilitate downstream process. We report the construction of three different expression vectors in order to obtain extracelullar L-ASNase from *D. chrysanthemi* using eukaryotic exrpression system. *asnB* gene from *D. chrysanthemi* was cloned in pJAG-s1 plasmid in fusion with endogenous signal sequence (SS), that addresses protein to bacterial periplasm, and with or without histidine tag (His). SuperMan$_5$ yeast strain was transformed with pJAG-s-*asnB* constructs in order to be able to express the recombinant protein. Aspartic acid β-hydroxamate method was applied for activity determination of L-ASNase recombinant in culture supernatants. When both SS and His-tag were removed (expression of mature protein), protein expression and secretion process were improved considerably compared to other constructions, indicating that for this gene, additional structures added to the recombinant protein may interfere with the expression, final enzyme activity and cell secretion. Purification processes are being executed.

Biography

Brian Effer is a PhD candidate at the University of La Frontera and University of São Paulo in Cell and Molecular Biology and Biochemical and Pharmaceutical Technology areas, respectively. He has published seven papers in reputed journals and has been serving as a referee in several journals.

breeiky@hotmail.com; b.effer01@ufromail.cl

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