

Study of *Saccharomyces cerevisiae* L-ASNase 1 Expression in *Pichia pastoris* *Glycoswitch* strain - potential use in the treatment of acute lymphoblastic leukemia (LLA)

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Introduction

Acute lymphoblastic Leukemia (ALL)

Mechanism of reaction of L- Asparaginase (EC 3.5.1.1)

Expression of Humanized L- Asparaginase

Currently, recombinant L-ASNase of bacterial origin derived from *E. coli* and *E. chrysanthemi* are produced

The L-ASNase of bacterial origin may be associated with side effects in patients due to their immunogenicity

Post-translational modifications (glycosylation) in yeast

An alternative is the expression of L-ASNase in yeast, due to glycosylation which could emulate the pegylation of the enzyme, avoiding the immunogenicity in the patients

Pichia pastoris yeast strain

We propose to perform the expression of L-ASNase 1 in the *Glycoswitch* strain of *P. pastoris* and to evaluate the humanized glycosylation induced in this organism

Objective

Construction of strains *Glycoswitch* His⁺ derived from *Pichia pastoris* with insertion of the sequence Pjag-s1_ ASP1.

Evaluation of the glycosylation and what effect it causes on the specific activity of Sc_ ASNase1

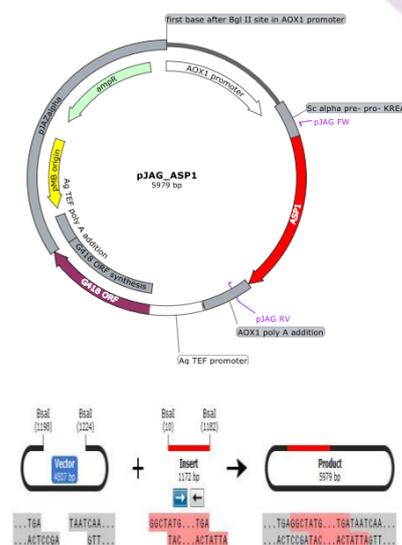
Purify the enzyme Sc_ ASNase1 and to evaluate the parameters Vmax, Km, Kcat, Kobs and efficiency time, under certain conditions of pH and temperature

Evaluate the modification of specific glycosylation sites in the structure of the enzyme Sc_ ASNase1 and its relation with certain physiological parameters

Test antitumor activity against *in vitro* leukemia cell lines

Results

Insertion of the ASP1 gene into pJAG-S1 vector



Verification of the open reading frame (ORF) of the sequence insert on pJAG-S1 vector

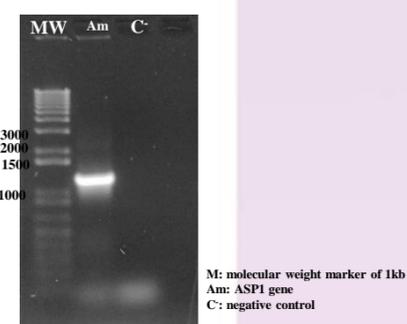
Protein sequence

Frame 1

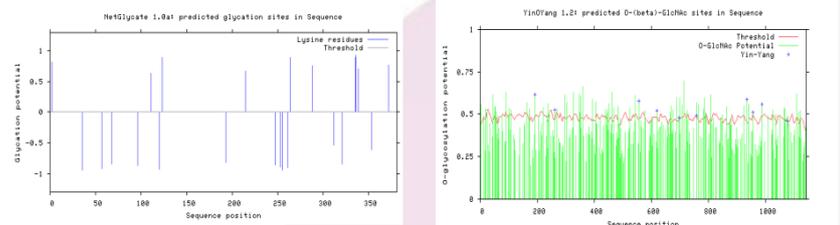
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ESTIDAGDVPFVFGSMRPSSTVSADGPMNLVQAICIASNPKSRGRVLY 200
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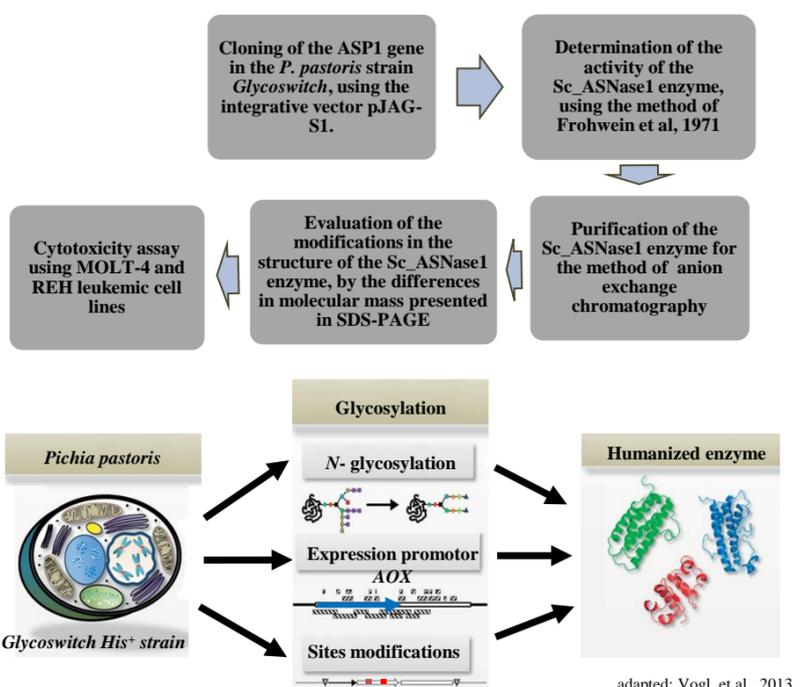
Verification of the insertion (ASP1 gene) in genome of the *Glycoswitch* strain



Prediction of possible glycosylated sites of ASNase1 enzyme



Materials and Methods



References

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