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Poster Presentation

Secretion of a hybrid K. *lactis*-A. *niger* β-galactosidase

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Background

The β-galactosidase from *Kluyveromyces lactis* is a protein with an outstanding biotechnological interest. The main limitation to its industrial production is the high cost associated with extraction and downstream processing due to its intracellular nature [1].

Secretion from yeast is an attractive method for producing many heterologous proteins both because of the facility with which genetic manipulations and fermentation can be carried out and because of the fidelity of posttranslational modifications. However, adding a signal sequence is not sufficient to lead recombinant proteins out of the cell: culture conditions play an important role, the wall acts as a molecular sieve but, moreover, structural determinants, present in the protein, may be required for targeting a protein to the medium [2].

In this work, we have constructed hybrid proteins between *K. lactis* β-galactosidase and *Aspergillus niger* β-galactosidase, added a signal peptide and analyzed the secretion and the properties of these new hybrid proteins.

Results

The highest levels of extracellular β-galactosidase were obtained when the segment corresponding to the five domain of *K. lactis* β-galactosidase was replaced by the corresponding five domain of the *A. niger* β-galactosidase. As medium composition can exert a profound effect on the yield of heterologous protein secretion in yeast, by influencing both cell growth and the specific rate of secretion [3] we examined hybrid β-galactosidase production and secretion on batch liquid cultures on several different media. Best results were obtained in a rich medium in which pH was maintained at 7.0, since pH values under 6.5 or above 7.5 cause a sharp decrease in *K. lactis* β-galactosidase activity [4]. In this condition the percentage of hybrid β-galactosidase secretion was in the exponential phase 2.2% and reached 16% of the total activity in the stationary phase.

Conclusion

One strategy for improving the secretion of heterologous proteins is through introducing structural modifications. A hybrid protein between *K. lactis* β-galactosidase and *A. niger* β-galactosidase was constructed that increase the fraction of enzyme reaching the growth medium. Moreover, we have improved secretion percentages by studying the influence of the culture conditions on heterologous hybrid β-galactosidase secretion.

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