# Construction of a flocculent brewer's yeast strain producing an *Aspergillus niger* β-galactosidase

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*KEY WORDS: Flocculation; Heterologous Protein; Cheese Whey; Lactose*

The yeast *Saccharomyces cerevisiae* is recognised to be an attractive host for the production of heterologous protein. However, the yeast expression system has a major drawback, that is, the rather modest intrinsic secretory capacity of *S. cerevisiae*. One way of improving heterologous protein production is to use high cell density systems being the flocculating yeast production system one of the most attractive. On the other hand, lactose is available in large amount as a waste of cheese processing systems. From the possible alternatives for lactose valorization, the alcoholic fermentation is undoubtedly an attractive one. The success of this process depends on the development of a continuous fermentation system using flocculating yeast strains.

The construction of a flocculent and non-flocculent brewer's yeast strain growing on lactose and secreting β-galactosidase is presented. A plasmid (pLD1) coding for an extracellular β-galactosidase of *Aspergillus niger* having as selective marker the *CUP1* gene which confers resistance to copper was constructed. This plasmid can be employed on the transformation of wild-type yeasts of commercial use, either for the production of β-galactosidase or for lactose consumption and production of ethanol. The results clearly show that the brewer's yeast transformed with pLD1 gains copper resistance, has β-galactosidase activity, grows on lactose medium and in the flocculent host strain, its flocculence ability is maintained. Previous results have been reported describing the partial secretion by *Saccharomyces cerevisiae* of β-galactosidase from *Aspergillus niger* by expression of the gene in a multicopy plasmid under control of the *ADH* promotor [1]. However, the plasmid employed uses an auxotrophic marker and can only be transformed into *ura*3 yeast strains. In industrial application stability can become a problem as the transformants will not be under selective pressure. Because copper sulphate is cheaper and more widely available than most drugs, the approach presented in this work is potentially more feasible.

Besides this application, the main achievment with this plasmid is the possibility of studying the production of heterologous proteins in flocculent yeast using two different strains, a flocculent and a non-flocculent, having the same parental strain and secreting the same heterologous protein.

[1] Kumar, V., Ramakrishnan, S., Teeri, T.T., Knowles, J.K.C., Hartley, B.S., Bio/Technology, 10, 82-85, 1992.

◆Lucília Domingues was supported by a grant from Praxis XXI (BD/11306/97). The financial support of ESF (European Science Foundation) and CIMO (Center for International Mobility) is greatly acknowledged.