GENETIC MODIFICATION OF *Pichia pastoris* FOR LACTOSE UTILIZATION AS A CARBON SOURCE

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Introduction. Lactose is the main component of whey, a residue from dairy industry which accumulates in large quantities and represents a serious environmental pollutant (1). Recently, the modification of specific phenotypes in a given organism has been achieved through metabolic engineering, which is the rational alteration of the genetic architecture of an organism (2).

In this work, we have engineered *Pichia pastoris* strains to use lactose as its sole carbon source.

Methods. A new *P. pastoris* strain was constructed by electroporation of the *P. pastoris* strain KM71pPIC9 with AvrII digested pLac plasmid, harboring two synthetic genes to be intracellularly expressed under the control of a constitutive promoter. The obtained strain was characterized by PCR using specific primers for the two cloned genes. This strain was grown in buffered minimal medium at 30ºC and 250 rpm with an initial OD_{600} of 0.1. Different single carbon sources were used: lactose (10 g/L), glucose (5 g/L) and galactose (5 g/L). The OD_{600} was measured every 6 h and the specific-growth rate (μ) was calculated. Samples were taken every 24 h for detecting both gene transcripts by RT-PCR and determining functionality of both enzymes by reaction with its substrate and the decrease of the lactose concentration in the cell-free culture medium by HPLC. The strain *P. pastoris* KM71pPIC9 was grown in lactose (10 g/L) as a control.

Results. Transformation of the *P. pastoris* KM71pPIC9 strain gave around 200 colonies, which were confirmed to present the correct genotype, since PCR analysis showed two bands of 1,977 bp and 3,265 bp, that correspond to the inserted genes, respectively. Growth kinetics (Fig. 1) showed that the μ *of* the new *P. pastoris* strain grown on lactose as a sole carbon source was two-fold higher (0.078 ± 0.003 h^{-1}) than the μ of the control strain (0.036 ± 0.003 h^{-1}), but two-fold lower than μ of the recombinant strain using glucose (0.155 ± 0.002 h^{-1}). Long-term growth of the new strain showed that the growth rate in lactose decreased 15-fold after 48 h, but continued growing for 144 h. The expression of both genes was confirmed by the amplification of transcript fragments. The lactose concentration decreased over time up to 50% at 48 h, and the intracellular beta galactosidase activity was 5,952 U/mg at the end of the culture (148 h), showing the functionality for both of the cloned genes.

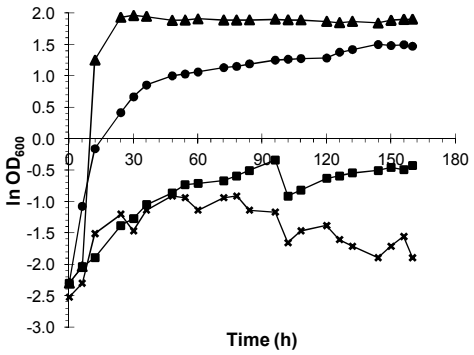

Fig.1. Growth kinetics of the new *P. pastoris* strain in different carbon sources. (▲) glucose 5 g/L; (■) galactose 5 g/L; (●) lactose 10 g/L; (x) control strain in lactose 10 g/L (n=3, C.V.≤ 10%).

Conclusions. The new *P. pastoris* strain could grow on lactose, indicating the functionally of the genetic design used for its construction, though this carbon source was less efficient than glucose or glycerol.

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