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VTT, Vuorimiehentie 3, PL 1000, 02044 VTT
puh. vaihde 020 722 111, faksi 020 722 4374

VTT, Bergsmansvägen 3, PB 1000, 02044 VTT
tel. växel 020 722 111, fax 020 722 4374

VTT Technical Research Centre of Finland
Vuorimiehentie 3, P.O. Box 1000, FI-02044 VTT, Finland
phone internat. +358 20 722 111, fax + 358 20 722 4374

VTT, Tietotie 2, PL 1000, 02044 VTT
puh. vaihde 020 722 111, faksi 020 722 7071

VTT, Datavägen 2, PB 1000, 02044 VTT
tel. växel 020 722 111, fax 020 722 7071

VTT Technical Research Centre of Finland, Tietotie 2, P.O. Box 1000, FI-02044 VTT, Finland
phone internat. +358 20 722 111, fax +358 20 722 7071

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Adaptive evolution of a recombinant lactose-consuming *Saccharomyces cerevisiae* strain

P. Guimarães[1], J. L. Parrou[2], V. Le Berre[2], J. Francois[2], J. A. Teixeira[1] and L. Domingues[1]

[1] Centro de Engenharia Biológica – Universidade do Minho, Braga, Portugal
[2] Centre de Bioingenierie Gilbert Durand, Institut National des Sciences Appliquees, Toulouse, France

In previous work, a recombinant *S. cerevisiae* flocculent strain (NCYC869-A3/T1, or simply T1) with the ability to express both the *LAC4* (coding for beta-galactosidase) and *LAC12* (lactose permease) genes of *Kluyveromyces lactis* was constructed (Domingues *et al.*, Appl Microbiol Biotechnol 51:621–626, 1999). The original recombinant obtained (T1) was able to metabolise lactose but slowly. Thus, it was subjected to an adaptation period, where the recombinant yeast was kept in liquid lactose medium, refreshed periodically. Cells collected after the adaptation process presented improved fermentative characteristics compared to the original transformant, namely higher growth rate and higher ethanol productivity. This evolved strain was named T1-E. The fermentative parameters (shake-flask cultivations with buffered lactose defined mineral medium) of strain T1-E are similar to *K. lactis* wild-type strain CBS2359 (NRRL-Y1140).

We aim at elucidating what happened during the process of adaptation/evolution that the yeast went through. The plasmid used for transformation (pKR1B-Lac4-1), which harbors a 13 kb region of the *K. lactis* genome including *LAC4* and *LAC12* genes, remained autonomous in the recombinant strain. Plasmid isolated from T1 (before adaptation) was identical to pKR1B-Lac4-1. However, we found that the plasmid isolated from T1-E carries a 1594 bp deletion (positions -518 to -2111 from the 5' end of *LAC4*) in the promoter region between *LAC4* and *LAC12* genes. This deletion may have improved the transcription of one or both of the genes, which may be the cause for the improved lactose consumption phenotype of the evolved strain. In lactose cultivations, the intracellular beta-galactosidase activity of strain T1-E is about 40 times higher when compared to T1. Moreover, the level of beta-galactosidase activity in strain T1-E is comparable to *K. lactis* CBS2359.

Microarray analysis showed increased expression of genes related with transposable elements in T1-E compared to T1, which reflects the selective pressure that the yeast suffered during the adaptation process. The transcriptome (*S. cerevisiae*) analysis did not revealed other important differences between T1 and T1-E.