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An evolved recombinant plasmid-carrying *Saccharomyces cerevisiae* strain presenting an improved lactose consumption phenotype.

Pedro Guimarães, Nelson Lima, José A. Teixeira, Lucília Domingues
Centro de Engenharia Biológica, Universidade do Minho, Campus de Gualtar, Braga, 4715-057, Portugal

In previous work, a recombinant *S. cerevisiae* flocculent strain (NCYC869-A3/T1) with the ability to express both the *LAC4* (coding for β-galactosidase) and *LAC12* (lactose permease) genes of *Kluyveromyces lactis* was constructed (Domingues *et al.*, Appl Microbiol Biotechnol 51:621-626, 1999). After transformation, the recombinant strain NCYC869-A3/T1 metabolised the same amount of lactose (10 g/L) regardless of the initial lactose concentration. However, after an adaptation period, where the yeast was kept in liquid lactose medium, refreshed periodically, the recombinant strain was able to completely metabolise 90 g/L lactose. This evolved strain is referred as NCYC869-A3/T1-E and we aim at elucidating what happened during the process of adaptation/evolution that the yeast went through. The plasmid KR1B-Lac4-1 (Sreekrishna and Dickson, Proc Natl Acad Sci USA 82:7909-7913, 1985) used for transformation, 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 NCYC869-A3/T1 (before adaptation) was identical to pKR1B-Lac4-1. However, we found that the plasmid isolated from NCYC869-A3/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.