sequences of the *Mucor* glyceraldehyde-3-phosphate dehydrogenase 1 gene (*gpd1*), were constructed. The *Mucor leuA* or *pyrG* genes were used as selection markers; they complement the leucine or uracil auxotrophy of the recipient *M. circinelloides* strain, respectively. Vectors were introduced alone ore in co-transformations to combine the isoprenoid genes. All transformants proved to be stable under selective conditions and some of them under non-selective conditions as well. Transformants were analyzed with hybridization and PCR techniques. Real-time PCR analysis revealed a relatively high copy number of the plasmids in the transformants and an unequal proportion of them in the co-transformants. Higher expression of the genes was also verified. The carotene production was analyzed by spectrofotometric, TLC and HPLC methods.

It has been found that *M. circinelloides* has β-carotene hydroxylase activity, therefore introducing the *crtW* gene may result in the production of several types of oxygenated β-carotene derivatives. Transformation with vector, containing the *crtW* gene under the control of *gpd1* promoter, was carried out (Papp et al. 2006) and co-transformations with the isoprene genes were also done. Changes in the carotenoid production due to expression of the *crtW* gene have been proven.

Integration the *crtW* gene into the *Mucor* genome was achieved by three different methods: homologous recombination with double crossing over, *Agrobacterium tumefaciens*-mediated transformation and restriction enzyme-mediated integration. The integration had been proven and analysed in several transformants by PCR, inverse-PCR, real-time PCR and hybridization techniques.

Carotene content of twenty one Zygomycetes strains was also analyzed. Some of them produced the same or higher amount of carotenoids than the wild type *M. circinelloides* or *B. trispora* strains. These strains were analyzed under different conditions, e.g. temperature, light and carbon source. For some of these strains, we started the development of new transformation systems that allows the direct selection of the transformants.

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Increased genetic stability of a rationally designed reduced-genome *Escherichia coli*

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In an attempt to engineer a simplified, core-genome *Escherichia coli*, we have reduced the wild-type K-12 MG1655 genome by making surgically precise scarless deletions (Pósfai et al. 2006). Genome reduction was achieved without compromising the basic metabolic circuits of the cells. The new strains, with genomes up to 22% smaller, were designed by bioinformatic comparative genomics of four *E. coli* strains to identify non-essential genes and recombinogenic, mobile or cryptic virulence sequences, as well as genes with unknown functions for elimination.

These so-called multi-deletion strains (MDS) have several attractive properties which can make them useful in a wide variety of biotechnological applications. One of the most important of these properties is the increased genetic stability of these strains which includes an increase in both genomic and plasmid stability. This work focuses on the quantification of these different aspects of genetic stability. This was done using novel methods we developed for calculating mutation rates (Fehér et al. 2006) as well as rates of recombination within the cells.

Removal of all mobile genetic elements from the *E. coli* genome resulted in a lower mutation rate because of the lack of insertion events. In addition, the genes of three so-called error-prone DNA polymerases (*polB*, *dinB* and *umuDC*) were deleted resulting in a lower point-mutation rate. The resulting strain has a mutation rate that is close to one order of magnitude lower than the wild-type.

In addition to the increased fidelity of replication, lentiviral expression vectors harbored within different MDS strains proved to be more stable than in other commonly used cloning strains (Chakiath and Esposito 2007). By developing a plasmid-based system to measure recombination rates, we were able to quantify this improved stability. The most stable of our strains has a recombination rate that is over five times lower than the wild-type.

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