Breeding of *Escherichia coli* based on colour

Commentary

C. Schmidt-Dannert, *et al.* (2000) Molecular breeding of carotenoid biosynthetic pathways.
***Nat. Biotechnol.* 18, 750–753**

These days, metabolic engineering allows the redirection of metabolic synthesis fluxes towards a desired product and the extension of existing pathways to achieve the synthesis of novel products. Cells have been engineered successfully to produce substances of commercial or scientific interest, both in primary and secondary metabolism. An emergent strategy is the 'molecular breeding' of biochemical pathways, that is, an *in vitro* evolution by mixing and matching of genes from different sources and different pathways, random mutagenesis, recombination and selection.

Schmidt-Dannert *et al.* demonstrate the capabilities of molecular breeding by manipulating *Escherichia coli* so that a multitude of carotenoids is produced, even though the (colourless) wild-type is non-carotenogenic. Besides the technological importance of carotenoids for food colourants and animal feed and nutrient supplements, these substances have recently attracted considerable interest because of their potential role in the prevention of cancer. Schmidt-Dannert *et al.* first transformed *E. coli* cells to express geranylgeranyldiphosphate synthase and phytoene synthase from *Erwinia uredovora* and phytoene desaturase from either *E. uredovora* or *Erwinia herbicola*. These transformed cells produced lycopene (showing an orange to orange-red colour) as the exclusive carotenoid. Using *in vitro* homologous recombination (DNA shuffling) of the genes that encode phytoene desaturase from the two *Erwinia* species, transformants were selected in which either tetradehydrolycopene (pink) or neurosporene (yellow) were produced in addition to lycopene. Similar recombination experiments were then performed with lycopene cyclase from the two *Erwinia* species in the different clones obtained in the first experiment. This led to clones with colours that varied from bright yellow-orange to purple-red. The carotenoids obtained were analysed using HPLC. The various clones turned out to synthesize, in addition to one or several of the above-mentioned acyclic carotenoids, the compounds β,β-carotene, β-zeacarotene, β,ψ-carotene, and torulene, which involve β-ionone rings. Synthesis of torulene is most spectacular because the microorganisms from which the genes were obtained do not produce this substance. Moreover, its synthesis proceeds along a different metabolic pathway in the organisms that produce it, such as red yeasts.

The work of Dannert *et al.* is a very impressive example of successful metabolic engineering. Metabolic pathways have been bred, in which one of the resulting pathways does not exist in nature. I can imagine that this work, however hard it was, was fun because of the curiousness to create colourful transformants. It is certainly worthwhile using similar experiments in teaching biotechnology.

Stefan Schuster
Department of Bioinformatics, Max Delbrück Center for Molecular Medicine, D-13092 Berlin-Buch, Germany.
(E-mail: schuster@hpbio1.biologie.hu-berlin.de)