Abstract

GFP is a protein which is extensively used in science. Thus the spectrum of the absorbance and excitation wavelength and also other properties like stability were changed during the years of research on it. Goal of this experiment was it to produce a red-shifted variant of GFPuv in the excitation wavelength with site directed random mutagenesis and *E. coli* as host. The resulting mutants showed an obvious excitation red-shift of around 80 nm. The fermentation was accidently done with the GFPuv wiltype version. Purified GFP was received with a concentration of 6.78 ± 4.2 mg/ml.

Introduction

During the whole experiment two different strains of *E. coli* were used. In detail these were the Top10 and the JM101 strains. The first strain is best suited for gene manipulation experiments and the second strain for industrial production, especially the growth on minimal medium (M9). Thus the first experiments were done in Top10 and later transferred into the second strain. A plasmid was taken containing a resistance to the antibiotic *Ampicillin* and containing a version of the *uvGFP* (green fluorescent protein) coding gene. With site directed mutagenesis this GFP gene was changed and later transferred into *E. coli*, resulting in a theoretical total of 262’000 mutated versions. The mutagenesis worked with pre-produced primers with random bases in a certain part (amino acids F64, S65 and S72) of the sequence, which were used in a PCR run in which many of this primers gave rise to unique sequences. For transferring the plasmids into the cells they were made competent, which means in detail that they were treated with Rubidium and a quick heat shock of 42°C. The analysis of the GFP mutant carrying *E. coli* was done with uv and blue light fluorescence analysis and the bacteria with highest visible fluorescence under blue light were chosen. The more detailed analysis later involved the use of DNA-sequencing, restriction mapping with *EcoRI*, *EcoRV* and *HindIII* separated with Agarose gel electrophoresis. The small scale GFP production and routine production analysis was observed with SDS-PAGE. For the test fermentation approximately 1.5 liters of *E. coli* were fermented with automatic feeding, pH balancing and O2 detection fermenter.

Discussion

The whole experiment was rather easy and uncomplicated. Interestingly the mutated sites gave rise to quite many red-shifted GFP variants. Around 5% of the produced colonies showed a red-shift of around 80 nm. Three of the four chosen mutants showed also a mutation in the DNA sequence and the fourth was a double mutant. The growth on M9 medium resulted in a growth rate of 0.50 h-1 which is in the approximated range. The bow of the growth curve in LB medium could be because of one component depleting which the cells could synthesize themselves. The analysis with the SDS-PAGE showed a rather small leaking of the GFP production, which could be avoided by taking another promoter. The *in silico* analysis showed many good candidates for mutation experiments, because of interactions with the fluorophore. From the chosen mutation sites one laid on the fluorophore, one close to it and one did not have any visible interactions with it. Reasons why the fermenter run produced the wildtype *GFPuv* are manifold possibly the best explanation is that during the picking of the colony somehow most of them have died and a wildtype contamination (possibly already in the medium) took over. Another reason could be that the wildtype was used for inoculation, although the fact that the correct plate showed signs of the hot wire.