STABLE-LIGHT PRODUCING *Escherichia coli*

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**SUMMARY**

Stable light production in *Escherichia coli* is achieved by cloning the genes encoding bacterial luciferase from *Vibrio harveyi*. To gain advantage of sensitive detection of light we transferred the genes under the control of a regulatable promoter system and searched for growth and buffer conditions where bacteria emitted stable light. Based on our findings an automated biosensor system can be developed to monitor the effects of biologically active compounds against stable-light producing bacteria.

**INTRODUCTION**

Biological sensors prepared with bacterial or eucaryotic cells provide a unique system to screen and measure a great variety of substances which lead to a response in cellular metabolism. Cells could be characterized as sacs of enzymes and their substrates covered with a semipermeable cover, the membrane. Often enzyme activity and stability are enhanced, for instance in *E. coli* where enzymes are in an "immobilized" form. Also cofactors and substrates of enzymes are present and continuously regenerated.

Bacterial luciferase is a mixed-function oxidase, which when reacting with its substrates, molecular oxygen, a long-chain aliphatic aldehyde and reduced form of flavin mononucleotide (FMNH₂), produces light at 490 nm (Hastings and Nealon, 1977). In the marine bacterium *V. harveyi* luciferase production is subject to so-called autoinduction where light emission is controlled by the density of the culture, but this does not happen in *E. coli* cloned with genes encoding luciferase from *V. harveyi* (Miyamoto et al., 1987). *E. coli* is supposed to have nearly the same capacity to reduce FMN by NAD(P)H:FMN oxidoreductase as *V. harveyi* (Baldwin et al., 1984). Only the exogenous aldehyde is needed to the bioluminescence system of *E. coli*.

We have studied the expression of the genes encoding luciferase from *V. harveyi* in *E. coli* (Karp et al., 1987a) and in *B. subtilis* (Karp et al., 1987b). In this work we aimed at a luminescence expression pattern in living *E. coli* such that all changes in light emission would be easy to interpret. By careful choice of the clones and cultivation conditions as well as the buffers used for measurements we found stable-light emission from *E. coli*. As an example of this luciferase based biosensor we report the use of stable-light emitting cells for detection of the presence of heavy metals like cadmium.

**MATERIALS AND METHODS**

*Bacterial strains and growth conditions*  
*E. coli* JM103 (Δ(lac-pro), thi, strA, supE, endA, shcB15, hsdR4, F'(traD36, proAB, lacF'ZΔM15)) (Vieira and Messing, 1982) was used as host for plasmids. Bacteria were grown in LB-medium (Maniatis et al., 1982) supplemented with ampicillin (100 µg/ml) either at 30°C or at 37°C. If needed IPTG (isopropyl-β-D-thiogalactopyranoside, Sigma) was added to 1 mM.
DNA manipulations
Standard in vitro genetic methods were employed (Maniatis et al., 1982) using enzymes from various commercial sources (Boehringer-Mannheim, Pharmacia).

Measurement of bioluminescence
The bioluminescence produced by E. coli cloned with genes encoding bacterial luciferase was measured with an automated LKB Wallac Luminometer 1251 (Turku, Finland) connected to a controlling device Olivetti M20 (Ivrea, Italy). The computer programs used to drive the luminometer are available from Pharmacia/Wallac Oy (Turku, Finland). The capacity of the luminometer carousel was 25 tubes and the minimum time interval for one tube to be measured was around two minutes. Ethanolic n-decanal (Sigma) solution was added to cells in final concentration of 0.001%. Measurements were done in Hank's balanced salt solution (HBSS) containing 0.14 g CaCl₂, 0.4 g KCl, 0.06 g KH₂PO₄, 0.1 g MgCl₂x6H₂O, 0.1 g MgSO₄x7H₂O, 8.0 g NaCl, 0.35 g NaHCO₃, 0.09 g Na₂HPO₄x7H₂O and 1.0 g glucose per liter, pH 7.4 supplemented with 0.1% gelatine.

RESULTS
Construction of pCSS108 and light emission profiles during cultivation
The genes encoding the luciferase alpha and beta subunits have been cloned from V. harveyi (Gupta et al., 1985). To obtain an expression of luminescence strong enough but not too harmful to cell metabolism we inserted these genes, under the control of the E. coli lac-promoter. A small Sall-PvuII -fragment containing luxA and B genes from plasmid pWH102 (Gupta et al., 1985) was cloned in pEMBL19 (Dente et al., 1983). Obviously the expression of lux genes in this construction was too high especially when lacP was induced with IPTG leading to rapid decay of the light emission in certain late growth phases (construction and data not shown). A similar but less dramatic decrease in light production was found when E. coli JM103[pCSS108] was grown at 37°C. The construction shown in Fig. 1 contained a longer 5 kb BamHI-BamHI -fragment from pWH102 in a corresponding plasmid pEMBL18 (Dente et al., 1983) inserted in the polylinker of the plasmid. E. coli JM103[pCSS108] was grown at 30°C and 37°C in L-broth containing ampicillin to find out suitable growth conditions with high enough light emission patterns (see Fig. 2). As can be seen from this figure the light emission dropped sharply at the beginning of stationary phase when bacteria were grown at the higher temperature. If lac-promoter inducer IPTG was added in the culture medium an even more pronounced drop was noticed (data not shown). When bacteria were grown at 30°C the bioluminescence continued to increase gradually till late logarithmic phase and stayed at that level for several hours.

Stabilization of light emission
To achieve stable light emission different conditions were examined. The substrate for luciferase reaction, n-decanal, was found to be optimal in a concentration...