The use of bacteriophage lambda P_L as a constitutive promoter

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The most widely used promoter for large-scale production of human therapeutic proteins is the λ P_L. The promoting activity of λ P_L is switched off at low temperature (28–30°C) in the presence of a clts gene that specifies a temperature-sensitive repressor but could be activated by heat induction (usually 42°C). The clts regulator can be located either on the host chromosome, on a second plasmid pRK248 compatible with the cloning vehicle, or on the vehicle itself.

In our laboratory, using the system based on the control of λP_L promoter by the pRK248, after a temperature shift from 30°C to 42°C, high expression levels of human growth hormone (hGH) were achieved, yields in general well above 1.5 μg/mL/A_{600}. However, attempts to express a related protein hormone (hPRL) based on the same protocol were not successful, providing 0.03 μ g/mL/A_{600} at most. Based on these results, and considering that hPRL is a thermo sensitive protein we decided to use strains lacking the pRK-248. Therefore we carried out expression studies, for each hormone, in presence or not of the repressor, cultivated under different temperatures.

Concerning the hGH results, at 42°C, no significant difference of expression was observed ($p>0.15$). In the presence of the repressor, the λ P_L promoter was almost totally repressed up to 37°C, while without the repressor, at 30°C, a quite high hGH secretion (1μ g/mL/A_{600}) was obtained. In the case of hPRL, at 37°C, utilizing the bacterial strain lacking the clts repressor, a high hPRL secretion level, 0.92 ± 0.10 μg/mL/A_{600} (n=6; CV=10.4%), was observed, i.e. approximately 30-fold higher than that obtained with the equivalent strain containing the repressor gene.

Since it has been reported in the literature that the lack of repressor could easily lead to plasmid loss, a study was carried out to determine the hPRL periplasmic yield in the strain lacking clts after two growth periods, corresponding to 10 and 50 *E. coli* generations. Yields respectively of 0.64 ± 0.05 and 0.78 ± 0.03 μ g hPRL/mL/A_{600} were obtained. The presence or not of antibiotic (amp) did not influence the specific expression yield, for at least 40 generations.

We concluded that these data open the way to the utilization of λ P_L as a constitutive promoter for increasing the expression of thermo sensitive proteins like hPRL.

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11434