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# Human Prolactin (hPRL) and Growth Hormone (hGH) distinct behavior under bacteriophage lambda $P_L$ promoter control

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## Background

When producing recombinant protein in *E. coli* for therapeutic use, it is desirable not only to obtain substantial amounts of it, but also make sure that potential contaminants such as antibiotics or inducing agents (isopropyl-beta-D-thiogalactopyranoside, IPTG or nalidixic acid) will not taint the final product. To prevent this shortcoming we can use expression systems where the promoter is activated by temperature shift, which denatures the controlling repressor protein cIts, allowing promoter activity. While in our hands hGH was successfully expressed and secreted in *E. coli* periplasm with yields in general well above 1 μg/mL/$A_{600}$, after a temperature shift from 30°C to 42°C [1], attempts to express a related protein hormone (hPRL) with basis on the same protocol were not successful, providing 0.03 μg/mL/$A_{600}$ at the most. Knowing that hPRL compared with hGH is a much more labile protein, we tried to obtain it from the same strain, but without the presence of the repressor protein and under optimized temperature conditions.

## Results

Human growth hormone periplasmic secretion in a bacterial host that has also been transformed with the plasmid pRK-248cIts, which contains the thermosensitive transcription repressor (cIts) gene [2,3] has been studied at different activation temperatures (Table 1). We can observe that the $\lambda P_L$ promoter is almost totally repressed up to 37°C, while at 42°C its derepression permits a useful hGH periplasmic secretion that is acceptable according to previously established parameters [1]. In Table 1 we also can see the results obtained in a set of different experiments observing that without repressor still a quite high hGH secretion (1 μg/mL/$A_{600}$) is obtained at 30°C while an apparently higher secretion is obtained at 42°C. Under the same conditions, considering the described expression vector into which the hGH gene has been substituted by the hPRL gene, still with the presence of cIts, an approximately 130–180-fold lower secretion level for this hormone is observed at 42°C. This led us to better study the behaviour of our hPRL-producing *E. coli* strains, either transformed or not with the plasmid pRK-248cIts. Considering not only that hPRL has been found a particularly labile protein but also that the bacterial periplasmic enviroment can even be more detrimental to protein stability, especially at high temperatures [4], it was decided to carry out a study on hPRL periplasmic secretion by "activating" at 30°, 35°, 37° and 42°C, with and without the presence of the repressor. The "activating" temperature of 37°C, and the bacterial strain lacking the cIts repressor, thus provided the highest hPRL secretion level, i.e. approximately 30-fold higher that those obtained with the equivalent strain containing the repressor gene. In Table 2 we can appreciate the statistical difference for prolactin yields obtained under different temperatures. As already observed for hGH (Table 1) the strain lacking the repressor gene is producing a significantly higher ($P < 0.001$) amount of hPRL, even at 42°C.

Since it has been reported that the lack of repressor could easily lead to plasmid loss [3], a study was carried out determining hPRL periplasmic yield in the strain lacking

**Table 1: hGH periplasmic secretion level activating at different temperature and utilizing hGH-secreting W3110 strains, with or without the repressor gene (cIts).**

| temperature | phGH-DsbA-λ$P_L$+ pRK-248 cIts (μg/mL/$A_{600}$ ± SD) | phGH-DsbA-λ$P_L$(μg/mL/$A_{600}$ ± SD) |
|---|---|---|
| 30°C | 0.01 | 1.0 ± 0.14 (n = 2) |
| 35°C | 0.02 | - |
| 37°C | 0,06 | - |
| 42°C | 1,31 ± 0.38 (n = 4) | 1.61 ± 0.11 (n = 3) |

**Table 2: hPRL periplasmic secretion level in *E. coli* W3110, at different temperatures utilizing a vector containing (phPRL-DsbA-cIts - λ$P_L$) and one not containing (phPRL-DsbA-λ$P_L$) the repressor gene.**

| temperature | phPRL-DsbA-λ$P_L$+ pRK-248 cIts (μg/mL/$A_{600}$ ± SD) | phPRL-DsbA-λ$P_L$(μg/mL/$A_{600}$± SD) | Statistical significance [a] |
|---|---|---|---|
| 30°C | 0.001 (n = 1) | 0.14 ± 0.02 (n = 6) | - |
| 35°C | - | 0.73 ± 0.07 (n = 5) | P < 0.001 |
| 37°C | 0.03 (n = 1) | 0.92 ± 0.10 (n = 6) | P < 0.01 |
| 39°C | - | 0.60 ± 0.12 (n = 8) | P < 0.001 |
| 42°C | 0.02 (n = 1) | 0.19 ± 0.05 (n = 4) | P < 0.001 |

[a] Student's T-test comparing each value to the previous one

cIts after two growth periods corresponding to 10 and 50 *E. coli* generations, obtaining 0.64 ± 0.05 and 0.78 ± 0.03 μg hPRL/mL/$A_{600}$ respectively. Also the presence or not of antibiotic (amp) did not influence the specific expression yield for at least 40 generations.

This same strain is being utilized for setting up a rapid and flexible feed batch fermentation in a laboratory bioreactor, obtaining up to now ~7 μg hPRL/mL with an optical density of 42.4 $A_{600}$.

## Conclusion

A relatively high hPRL periplasmic secretion (up to 0.9 μg/mL/$A_{600}$), never reported before, has been obtained by constitutive expression of the unrepressed λ$P_L$promoter, at 37°C. The expression level is approximately 10-fold higher than that obtained in previous work [5] by using an IPTG-activated tac promoter. We can conclude that these data open the way to the utilization of *E. coli* instead of insect or mammalian expression systems for the production of an authentic and highly homogeneous hPRL.

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