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BIOACTIVE AND PROPERLY FOLDED PROLACTIN IS OBTAINED IN *E. COLI* IN A PILOT BIOREACTOR AT 37°C BY CONSTITUTIVE EXPRESSION BASED ON THE UNREPRESSED ?P$_L$ PROMOTER

Oliveira TL; Heller SR; Arthuso FS; Sousa JM; Bartolini P; Soares CRS
Centro de Biotecnologia, IPEN-CNEN/SP

crsoares@ipen.br

Human prolactin (hPRL) is a polypeptide hormone of molecular mass 23,000 with a single chain of 199 residues and three disulfide-bonded loops. It is one of the most versatile hormones in terms of biological actions, more than 100 different effects having been documented. Our research group has been the first to obtain useful secretions of hPRL in *E. coli* periplasmic space but, differently from human growth hormone (hGH), the utilization of a temperature controlled ?P$_L$ promoter only provided extremely low amounts of the hormone (~0.03µg/mL/A$_{600}$). We describe the set up, in shake flask cultures, of a constitutive hPRL expression based on the unrepressed ?P$_L$ promoter, under optimized temperature conditions (37°C). This way useful specific expression levels of about 0.9 µg hPRL/mL/A$_{600}$ were obtained. Since no industrial production of hPRL in *E. coli* has ever been described, we also set up pilot scale cultivation conditions that provided a useful specific expression of 1.0µg hPRL/mL/A$_{600}$ at an optical density of 35 A$_{600}$. Its purification and characterization confirmed that the protein is highly monomeric, authentic and properly folded, with a biological activity of ~51 IU/mg, not significantly different from the first WHO Reference Reagent (97/714).

Key words: *E.coli*, fed batch fermentation, human prolactin, ?P$_L$ promoter

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