Recombinant expression of Leptin hormone in *Pichia pastoris*, as a biosimilar option for orphan disease treatment

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Introduction

An orphan disease is chronically debilitating, severe, life-threatening, and prevalence of less than 1 per 5,000 people. The cause of orphan diseases is still unknown. However, they have a common factor: a genetic disorder that can affect and reduce life quality, physical capabilities, and life expectancy (1). Obesity due to congenital Leptin deficiency is a rare disease, defined as an erroneous mutation in the leptin gene (*Lep*), and associated with a morbid obesity phenotype and significant alterations in metabolic and immune functions (2). Leptin is an adipokine that plays an essential role in physiological functions such as regulating body weight and energy balance, reproduction, glucose homeostasis, tissue remodeling, and other functions of the endocrine and immune systems (3). Other specific conditions caused by leptin deficiency are lipodystrophy syndrome, hypothalamic amenorrhea (associated with excessive exercise or ovarian failure), and anorexia nervosa (4-6). Leptin has 167 amino acids, 16 kDa, four alpha-helix, and a disulfide bridge (7). The orphan disease congenital leptin deficiency affects few people but demands a costly treatment. Currently, the disease is effectively treated with recombinant Leptin obtained through biotechnological genetic engineering processes; however, it is not produced in Colombia. This project aims to develop a genetically modified organism capable of heterologously expressing the Leptin hormone in the *Pichia pastoris* yeast.

Methodology

The Leptin gene was designed *in-silico*, considering the preferential codons used for Pichia pastoris. The Leptin was synthesized and introduced into a cloning plasmid, then digested with *Xho*I and *Not*I enzymes. The obtained insert (Leptin gene and cohesive sites) was ligated within the expression plasmid pPICZ-**α** –digested with the same enzymes–. Afterward, we obtained the plasmid pPICZ-**α**-Leptin, used to transform *E. coli* XL1Blue. Some colonies were evaluated by colony PCR to verify the Leptin gene presence. One positive colony was amplified to purify its plasmid and sequencing. We transformed *P. pastoris* GS115(Mut+) with this corroborated plasmid, obtaining a recombinant yeast grown in BMGY and induced in BMMY media (Figure 1).

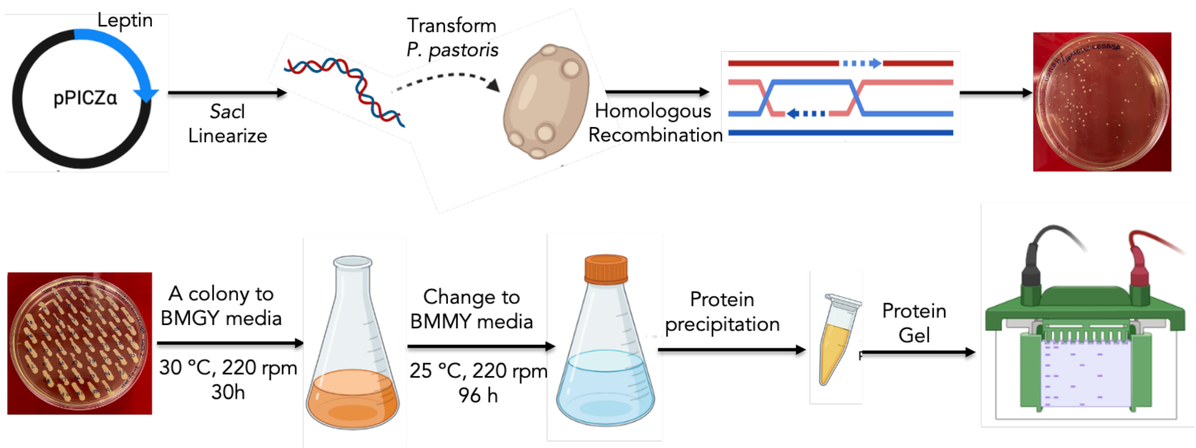

Figure 1. Graphical procedure to obtain recombinant leptin.

Results

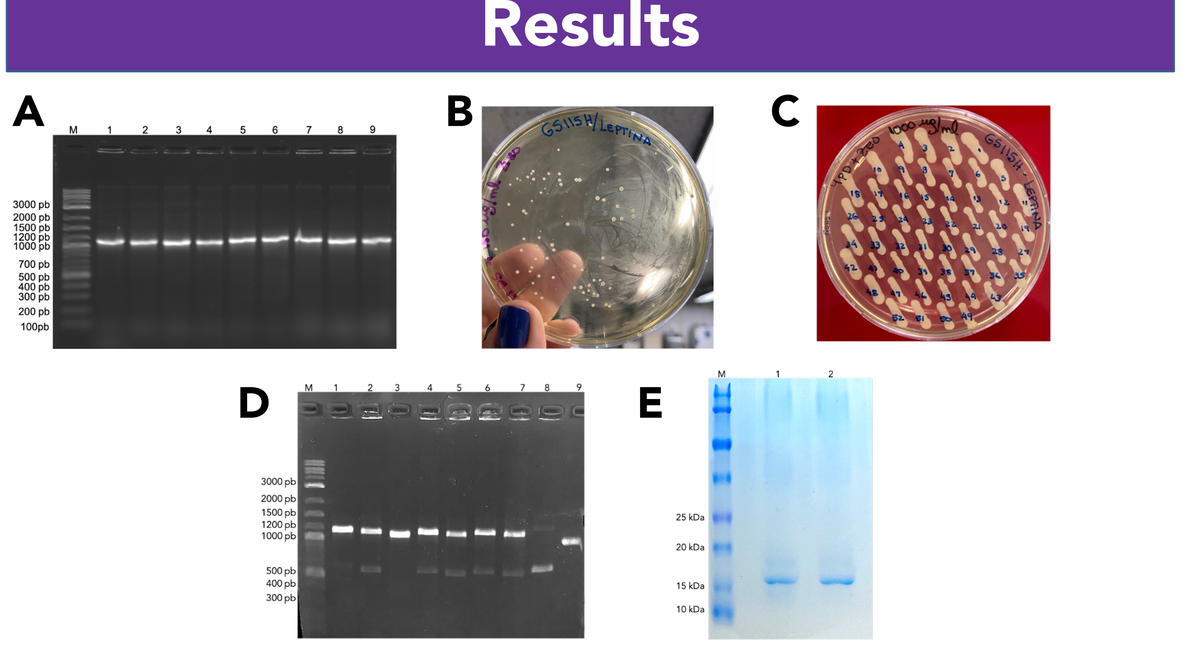

Figure 2. A. Colony PCR result. M: base pair marker; Wells 1 to 9: colonies from the transformation of *E. coli*XL1Blue cells with plasmid pPICZ-**α**-Leptin. Amplification expected 1000 bp. **B.** Yeasts resulting from recombination (pPICZ-**α**-Leptin restricted with *Sac*I + *P. pastoris* GS115H). **C.** Recombinant yeasts grown in 1,000 µg/mL of the antibiotic Zeocin. **D.** Recombinant yeast colony PCR: M: base pair marker; Wells 1 to 7: recombinant yeast colonies; Well 8: empty *P. pastoris* GS115H ; Well 9: plasmid pPICZ-**α**-Leptin. **E.** SDS-PAGE (4/15%) of samples from the extracellular culture of 2 recombinant yeasts purified by Ni affinity column (IMAC). A band with 90% purity and the expected apparent size for the hormone leptin (~17kDa) is observed.

Recombinant Leptin was purified from the supernatant by Ni-resin (8µg/µL). SDS-PAGE analysis confirmed the expression and integrity of a unique ~17 kDa band corresponding to Leptin. We still need to optimize the culture conditions, and Leptin production could be used as a medical treatment for rare diseases.

Conclusions

Leptin activity studies in the human body have demonstrated the importance of this protein in regulating fundamental activities of the endocrine and immune systems and in the body's energy balance. It has been shown that its deficiency can have serious consequences, causing diseases that must be treated with the hormone, which in many cases is inaccessible due to its high cost.

Obtaining recombinant leptin in the *P. pastoris* system proposed in this work, we wish to reach high protein yields and evaluate its biological activity. The aim is to establish a path that favors and allows the development of the biosimilar drug and contributes to improving the life quality of those who need the medication and do not have access to it.

References

1. Schieppati A, Henter J-I, Daina E, Aperia A. Why rare diseases are an important medical and social issue. The Lancet. 2008;371(9629):2039-41. https://doi.org/10.1016/s0140-6736(08)60872-7.

2. Bluher S, Shah S, Mantzoros CS. Leptin deficiency: clinical implications and opportunities for therapeutic interventions. J Investig Med. 2009;57(7):784-8. https://doi.org/10.2310/JIM.0b013e3181b9163d.

3. Dagogo-Jack S. <Leptin_ Regulation and Clinical Applications.pdf>. Springer. 2015. https://doi.org/10.1007/978-3-319-09915-6.

4. Wasim M, Awan, F. R., Najam, S. S., Khan, A. R., & Khan, H. N.Khan, H. N. Role of Leptin Deficiency, Inefficiency, and Leptin Receptors in Obesity. Biochemical Genetics. 2016;54(5):565-72. https://doi.org/10.1007/s10528-016-9751-z.

5. Jean L Chan CSM. Role of leptin in energy-deprivation states normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. The lancet. 2005;366:74-85. https://doi.org/10.1016/S0140-6736(05)66830-4

6. Reitman ML, Arioglu, E., Gavrilova, O., & Taylor, S. I. Lipoatrophy Revisited. Trends in Endocrinology & Metabolism. 2000;11(10):410-6. https://doi.org/10.1016/S1043-2760(00)00309-X

7. Denisse Goldenberg Jls, María isabel Hodgson, Víctor A. Cortés. Novel physiological and therapeutic implications of leptin. Rev Med Chile. 2014;142:738-47. http://dx.doi.org/10.4067/S0034-98872014000600008

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