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Development of a Recombinant Human Insulin Formulation Using Yeast Expression System: A Biotechnological Approach

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Abstract

Recombinant insulin remains one of the most impactful biotechnological products in medical history. This study focuses on the development and optimization of a cost-effective recombinant human insulin formulation using Pichia pastoris as the host organism. The expression vector containing the human insulin gene was introduced into the yeast strain, followed by protein expression, purification, and preliminary stability assessment. High-yield expression, functional folding, and biological activity were confirmed through ELISA and glucose uptake assays in vitro. The yeast expression system proved to be scalable, economical, and effective for producing bioactive insulin suitable for therapeutic applications. This research provides a foundation for future large-scale biosimilar production.

Keywords: recombinant insulin, pichia pastoris, pharmaceutical biotechnology, biosimilars, protein expression, metabolic assay

Introduction

Biopharmaceuticals, especially recombinant proteins, have revolutionized treatment modalities across chronic and acute diseases. Insulin, a hormone crucial for glucose metabolism, is a prime example of a successful recombinant therapeutic. Initially derived from porcine or bovine sources, the introduction of recombinant DNA technology enabled human insulin production, improving efficacy and reducing immunogenicity.

Traditional production systems use *Escherichia coli*, but these require complex protein refolding and lack post-translational modifications. Yeast systems, particularly *Pichia pastoris*, offer a eukaryotic environment, high

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cell density fermentation, and cost-effective scalability. This study explores the expression of human insulin in *Pichia pastoris*, aiming to develop an efficient production process with downstream therapeutic potential.

Materials and Methods

1. Strain and Plasmid Preparation
The human insulin gene was synthesized and cloned into the pPICZαA vector under the control of the AOX1 promoter. The recombinant vector was transformed into competent *Pichia pastoris* X-33 cells via electroporation.
2. Culture Conditions
Transformed cells were selected on Zeocin-containing YPD agar. Positive colonies were screened in BMGY medium and induced in BMMY medium with 1% methanol every 24 hours for 96 hours.
3. Protein Extraction and Purification
Secreted insulin was recovered from the culture supernatant, concentrated, and purified using affinity chromatography (His-tag purification system), followed by dialysis.
4. Expression Validation
SDS-PAGE and Western blot were used to confirm the presence of recombinant insulin. ELISA was used for quantification.
5. Bioactivity Assay
Biological activity was assessed through glucose uptake assays in cultured 3T3-L1 adipocytes treated with recombinant insulin.
6. Stability Testing
The purified insulin was stored at 4°C, 25°C, and 40°C for 30 days and analyzed periodically for degradation and activity loss.

Results

The recombinant insulin gene was successfully integrated into the *Pichia pastoris* genome. High levels of expression were observed after 96 hours of methanol induction. SDS-PAGE revealed a protein band consistent with the expected molecular weight of insulin (~6 kDa), and Western blot confirmed its identity.
ELISA quantification demonstrated expression levels of approximately 20 mg/L in optimized conditions. Bioactivity assays revealed that the yeast-derived insulin significantly enhanced glucose uptake in adipocyte cultures, comparable to a commercial standard.
Stability testing indicated minimal degradation at 4°C and 25°Cover 30 days, while storage at 40°C led to partial degradation after two weeks.

Discussion

The results affirm that *Pichia pastoris* is a viable host for the expression of bioactive recombinant insulin. Compared to bacterial systems, yeast offers advantages such as proper protein folding, secretion, and simplified purification protocols. The achieved expression levels are promising, although optimization of fermentation conditions could further improve yield.
The glucose uptake assay confirms that the insulin retains its functional activity, suggesting its suitability for therapeutic applications. The stability profile supports its feasibility for storage and transport, critical factors in pharmaceutical logistics.
This work lays the groundwork for future development of insulin biosimilars in low-cost settings, with implications for global diabetes care accessibility.

Conclusion

A functional, stable, and economically viable recombinant insulin formulation was successfully developed using *Pichia pastoris*. The yeast-based expression system demonstrated high yield and biological efficacy, providing a promising platform for scalable biosimilar production in pharmaceutical biotechnology.

References

1. Walsh, G. (2018). *Biopharmaceuticals: Biochemistry and Biotechnology*. Wiley-Blackwell.
2. Cereghino, J.L., & Cregg, J.M. (2000). Heterologous protein expression in the methylotrophic yeast *Pichia pastoris*. *FEMS Microbiology Reviews*, 24(1), 45–66.
3. Steiner, D.F., & Oyer, P.E. (1967). The biosynthesis of insulin and a probable precursor of insulin.

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Proceedings of the National Academy of Sciences, 57(2), 473–480.

4. Li, H., Sethuraman, N., Stadheim, T.A., et al. (2006). Optimization of humanized IgG production in *Pichia pastoris*. *Biotechnology Progress*, 22(4), 1090–1098.
5. Singh, R.S., & Pandey, A. (2021). Recombinant protein expression in microbial systems. *Current Biotechnology*, 10(1), 1–12.
6. World Health Organization. (2021). *Global report on diabetes*. Geneva: WHO Press

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