Medium optimisation for the production of food protein in *Yarrowia lipolytica*, towards economic feasibility at industrial scale

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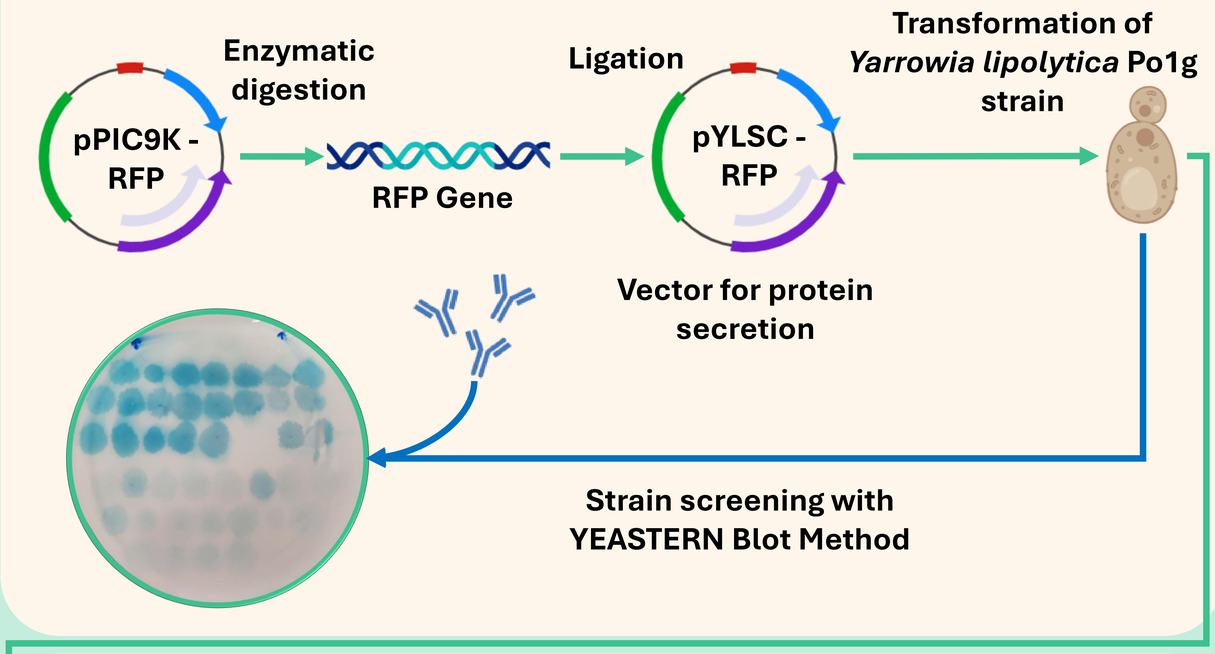
1. Introduction

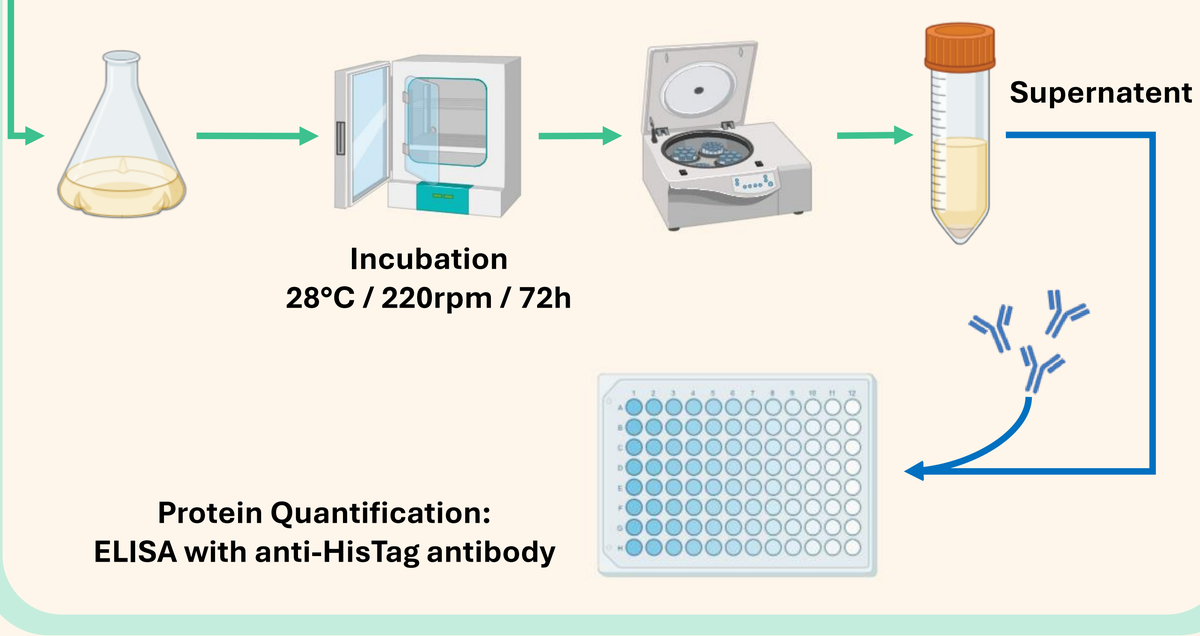
The growing demand for dietary protein presents challenges for environmentally **sustainable food production** [1]. **Precision fermentation** offers a sustainable and innovative solution by employing engineered micro-organisms to produce **animal-derived proteins** for use in **food applications** [2]. However one of the main challenges of this technology lies in controlling the costs associated with upstream processing (USP) and downstream processing (DSP). Traditionally, protein expression in yeast relies on **complex organic media** like YPD**.** However, these media introduce batch-to-batch variability, increase production costs, and limit process reproducibility at industrial scale. Chemically defined **mineral media** like YNB offer a promising alternative by providing controlled nutrient composition, reducing contamination risks, improving consistency, and significantly lower raw material costs [3]. This study aims to optimize the economical performance of defined mineral media [4] compared to a traditional organic-rich one, using a ***Yarrowia lipolytica* Po1g strain,** producing a **recombinant food protein (RFP)** by secretion.

2. Materials and Methods

Vector construction and strain transformation



Protein production and quantification



3. Results

3.1 Media Screening

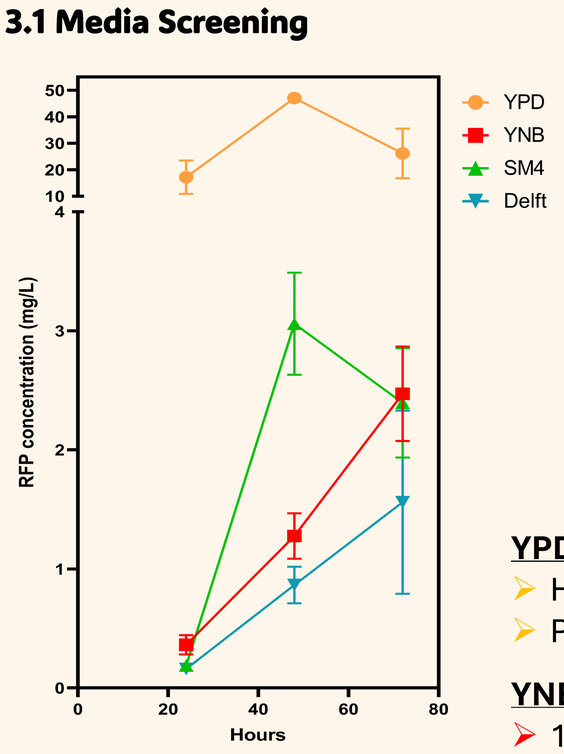


Figure 2: Media screening for the production of RFP

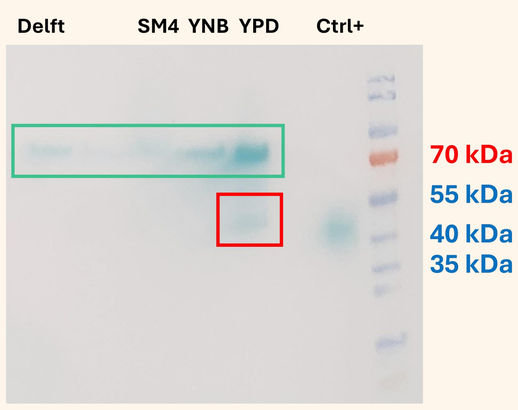


Figure 1: Western Blot of RFP production in different media

YPD:
- High production level
- Possible proteolysis after 72h

YNB:
- 1.28 mg/L at 48h
- **5.52 €/L**

SM4:
- 3.06 mg/L at 48h
- **9.54 €/L**

3.2 Media Optimisation

- Plackett-Burman (A/P) design of experiments

Figure 3: Mineral compounds with a positive or negative influence of the production of RFP

3.3 Potassium, Phosphate and Calcium sources

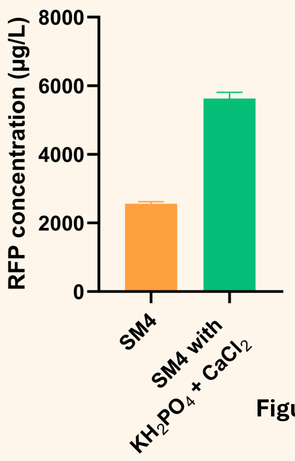


Figure 4: RFP Production in SM4 with different sources of potassium, phosphate and calcium

- In SM4:
  - K_2SO_4: ~ 280 €/kg
  - H_3PO_4: ~ 80 €/kg
  - $CaSO_4$: ~ 130 €/kg
- Replacement with lower-cost compounds:
  - $KH2PO_4$: ~ 90 €/kg
  - $CaCl_2$: ~ 50 €/kg

3.4 Carbon and Nitrogen sources screening

- Production yield is **maintained in SM4opt** after the DOE results
- Production yield is **increased with glucose** and **maintained with glycerol**, NH_4 and urea

Figure 5: RFP production in optimised SM4 media with different carbon and nitrogen sources

4. Discussion and Prospects

- Successfully optimized SM4 medium composition, reducing the cost from **9.54 €/L (SM4)** to **4.82 €/L (SM4opt)**, making it more cost-effective than YNB
- **Further work will focus on**:
  - Optimizing mineral concentrations to further reduce costs and improve production yields;
  - Integrating low-cost co-products to enhance both economic and environmental sustainability;
  - Validating the optimized medium in bioreactor cultivations to assess process scalability and industrial feasibility

5. References

[1] Eastham, Jane, et Annette Creedon. « Food Losses, Food Waste, and beyond in Food Supply Chains: Retaining Optimum Nutrient Density ». *Food Frontiers* 4, nº 3 (2023): 971-79. https://doi.org/10.1002/fft2.271.
[2] Eastham, J. Lucas, et Adam R. Leman. « Precision Fermentation for Food Proteins: Ingredient Innovations, Bioprocess Considerations, and Outlook — a Mini-Review ». *Current Opinion in Food Science* 58 (août 2024): 101194. https://doi.org/10.1016/j.cofs.2024.101194.
[3] Gasmi, Najla, Atef Ayed, Jean-Marc Nicaud, et Héla Kallel. « Design of an Efficient Medium for Heterologous Protein Production in Yarrowia Lipolytica: Case of Human Interferon Alpha 2b ». *Microbial Cell Factories* 10, nº 1 (2011): 38. https://doi.org/10.1186/1475-2859-10-38.
[4] Singh, Vineeta, Shafiul Haque, Ram Niwas, Akansha Srivastava, Mukesh Pasupuleti, et C. K. M. Tripathi. « Strategies for Fermentation Medium Optimization: An In-Depth Review ». *Frontiers in Microbiology* 7 (janvier 2017). https://doi.org/10.3389/fmicb.2016.02087.