**The quest for a cell factory for the production of recombinant proteins: *Pichia pastoris* Vs *Yarrowia lipolytica*.**

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**Highlights**

* *P. pastoris* and *Y. lipolytica* were compared for the production of recombinant proteins
* Calb lipase was used as a case report
* Protein productivity was higher in *Y. lipolytica* despite a lower gene expression level
* The lower productivity in *P. pastoris* is due to proteasome degradation of CalB

**1. Introduction**

The production at industrial scale of recombinant proteins (rProt) is of increasing economic importance. Among the different microbial chassis that has been developed for that purpose, yeasts are regarded as the preferred option for the production of recombinant enzymes and therapeutic proteins. The main advantage of yeasts over bacterial systems such as *Escherichia coli* relies on the possibility to obtain posttranslational modified proteins in the culture supernatant at grams per liter. Historically, *Saccharomyces cerevisiae* has been used as the reference eukaryotic chassis, however it suffers several drawbacks such as low protein productivity, overflow metabolism or hyperglycosylation phenomenon. Moreover, it is less metabolically adapted to catabolize raw carbon and nitrogen sources that are nowadays increasingly considered as nutrients in bioprocesses. The non-conventional yeasts *Pichia pastoris* and *Yarrowia lipolytica* are considered as realistic alternatives to *S. cerevisiae* for rProt synthesis. They both combine advantages of growing at high cell density, to produce and secrete rProt at high yield and to have low nutritional requirements, allowing thus to grow them on raw materials or industrial byproducts. Although these two yeasts are well-established cell factories for rProt synthesis in industry, no direct comparison of their performances has been reported so far. Here, we report such a comparison using the CalB lipase from *Candida antartica* as a model protein.

**2. Methods**

The codon optimized gene sequence of CalB was cloned under the control of strong inducible promoters. For *Y. lipolytica*, the hybrid promoter pEYKA3B inducible by erythritol was used in combination with a EYK1*ko* recipient strain that is unable to metabolize erythritol. For *P. pastoris*, the widely used promoter pAOX1 was used in combination with a MutS recipient strain (methanol low consumption). The resulting strains were then grown in bioreactors in optimized conditions and parameters such as cell growth, gene expression, carbon uptake rates and extracellular lipase activity were monitored over time.

**3. Results and discussion**

*Y. lipolytica* performances were by far superior in terms of cell growth rate and rProt synthesis. Compared to *P. pastoris,* it grew faster, at higher cell density and cells used carbon source more efficiently. The maximal lipase activities were equal respectively to 5540 and 1066 U/mgDCW for *Y. lipolytica* and *P. pastoris*, respectively, representing thus more than a 5-fold increase. Surprisingly, *P. pastoris* showed a significantly higher level (5-fold) of CalB gene expression as demonstrated by qRT-PCR. Several hypotheses has been formulated and tested to explain the reasons behind the observed differences. The lower protein productivity in *P. pastoris* was found related to the intracellular protein degradation by the proteasome complex.

**4. Conclusions**

From this study, *Y. lipolytica* appears a more efficient cell factory that is in addition not related to the utilization of flammable methanol as inducer.