**Erythritol-inducible promoter efficiently triggers lipase CalB production in bioreactor.**

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**Highlights**

* Three types of erythritol-inducible promoters were compared to *pTEF*.
* *pEYKA3B* and *pHu8EYK* led to 3-fold higher CalB expression than *pTEF*.
* *pEYKA3B* led to 3-fold higher CalB activity than *pTEF*.
* Glycerol 2-L fed-batch enabled consequent *pEYKA3B*-driven CalB production.

**1. Introduction**

The non-conventional yeast Yarrowia lipolytica is increasingly used as an alternative cell factory for recombinant protein production. Although efficient molecular tools have been developed for this host, there is still a lack of versatile regulated promoters. Such promoters would allow to modulate the detrimental metabolic load inherent to recombinant gene expression [1]. Current regulated promoters respond to hydrophobic inducers, dispersed in the culture medium as an emulsion technically sensitive to maintain, particularly at large scale. Recently, promoters from the erythritol kinase *EYK1* gene [2] and the erythritol dehydrogenase *EYD1* gene [3] have been isolated and shown to be induced by erythritol, a hydrophilic substance. Series of hybrid promoters have been developed from upstream activating sequences (UAS) of *pEYK1*, *pEYD1*, and other promoters, using fluorescent reporter proteins in small-scale cultures [2] [3]. Here we report on the comparison of three types of erythritol-inducible promoters (native, based on *UAS* from *pEYK1*, based on *UAS* from another promoter) and the subsequent selection of the most suitable promoter for proof-of-concept production of the industrial lipase B from Candida antarctica (CalB) in 2-L bioreactor.

**2. Methods**

CalB expression was driven by promoters *pTEF*, native *pEYD1*, synthetic or *pEYKA3B* (*pEYK1* + 3 *UAS1* of *pEYK1*) and *pHu8EYK* (*pEYK1* + 8 *UAS1* of *pXPR2*). The host strain was optimized for recombinant lipase production, with deletions of alkaline extracellular protease *XPR2*, of extracellular lipases *Lip2*, *Lip7*, *Lip8*, and of *EYK1* gene (to use erythritol as a free inducer, no longer consumed by the cells).

Promoter comparison was based on triplicate 48h cultures in 2Mag mini bioreactors (10 mL working volume). qPCR and lipase activity assays were performed to assess promoter efficiency at gene expression and protein production level, respectively. Proof-of-concept cultures were realized during 72h in duplicate in a 2-L Sartorius bioreactor operated in fed-batch mode, with increasing glycerol feed values (0.45; 0.9; and 1.35 g.L-1.h-1, for 24h each). Lipase activity assays, SDS gel and carbon source HPLC assays were performed on culture supernatants.

The culture medium contained glycerol (10 g/L for 2Mag cultures, 1 g/L initially plus glycerol feed for 2-L cultures) as a main carbon source and erythritol (10 g/L) as an inducer.

**3. Results and discussion**

Erythritol-inducible promoters *pEYKA3B*, *pHu8EYK*, and *pEYD1* were compared to the strong constitutive promoter *pTEF* for CalB expression and production. At the expression level, *pEYKA3B* and *pHu8EYK* showed a 3-fold higher response than *pTEF*, while *pEYD1* presented the same behavior as *pTEF*. At the production level, *pEYKA3B* delivered a 3-fold higher extracellular CalB activity than *pTEF*, in accordance with qPCR results. *pHu8EYK* and *pEYD1* led to lower CalB activity levels. In consequence, *pEYKA3B* was selected for larger-scale experiments.

In previous studies, the induction levels of *pEYK1*-derived promoters were shown directly correlated with erythritol concentration in the culture medium, and erythritol cellular uptake appeared reduced in the presence of glycerol in the culture medium [2] [4]. Here, reactor cultures were operated in fed-batch mode to allow progressive glycerol feed at 3 increasing concentrations. In these conditions, glycerol accumulation in the culture medium was prevented, while providing sufficient energy for cell growth and CalB synthesis. Hence, during 2-L reactor cultures, CalB was accumulated in the culture medium, reaching a final value of about 900 U/gDCW. SDS gel analysis proved CalB adequate size and integrity in the culture supernatant.

**4. Conclusions**

Among the three types of erythritol-inducible promoters examined in the present study, the promoter *pEYKA3B* based on 3 *UAS1* from *pEYK1*, stood out with high CalB expression and production levels. Production of lipase CalB in fed-batch bioreactor demonstrated the adequacy of *pEYKA3B* and more generally erythritol-inducible promoters for recombinant protein production.

**References**

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