J Bacteriol Parasitol 2016, 7:6 (Suppl)
http://dx.doi.org/10.4172/2155-9597.C1.026

The *pPOX2* and *pLIP2* regulation in response to carbon source in the yeast *Yarrowia lipolytica*

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The non-conventional yeast *Yarrowia lipolytica* has been extensively used to produce recombinant proteins for various biotechnological applications. Actually, more than 110 proteins from human, plants and bacterial systems have been successfully expressed and produced in Y*arrowia lipolytica*. However, understanding the regulation of the promoters used to drive heterologous gene expression is a key parameter in the development of an efficient process. In this study, the regulation of the promoter of acycl-CoA oxidase gene 2 (*pPOX2*) and of the extracellular lipase 2 (*pLIP2*) were considered in regard to the medium composition and to the carbon source used (glucose, glycerol, oleic acid). Induction levels of promoters were measured using a reporter system based on a red fluorescent protein (DsRed). Specific fluorescence measurement revealed that *pLIP2* is more strongly induced than *pPOX2*, especially in complex medium. Interestingly, higher levels of induction were obtained when a combination of glucose and oleic acid was used as carbon source compared to an oleic acid based medium. In order to define the optimal ratio of glucose/oleic acid to be used, several ratios of carbon sources have been tested for their induction potential. A high induction level of *pLIP2* was obtained when oleic acid fraction in the culture medium was in the range of 0.6-0.9 cmol. Interestingly, relative fluorescence was increased slightly in this range by a factor of 18% compared to the use of 100% oleic acid. This result suggests that glucose can be considered as the most promising co-substrate to enhance recombinant protein production under *pLIP2*. Nonetheless, glycerol can replace partially oleic acid to express heterologous protein under *pPOX2*. Thus, the use of glycerol permits to lower the process cost but it also opens new perspectives for glycerol based microbiological processes. In conclusion, this work provides alternative strategies to enhance heterologous protein production in *Yarrowia lipolytica* which increase its interest as a promising recombinant expression system.

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