EXPRESSION LEVELS OF GENES INVOLVED IN THE PRODUCTION AND SECRETION OF A RECOMBINANT PHYTASE IN *Pichia pastoris* AND CORRELATION WITH THE PHYSIOLOGICAL ACTIVITY OF THE HOST AND THE PRODUCTION LEVEL OF THE RECOMBINANT PRODUCT

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Introduction. Information about expression levels of genes involved in the production and secretion of a recombinant protein could be useful for understanding how the host responds favorably or negatively to the production of a heterologous protein.

In this work, we evaluated the expression levels of genes involved in the production and secretion of the phytase FTEII in *Pichia pastoris,* and correlated with the physiological activity of the host and the production level of the recombinant product.

Methods. A *P. pastoris* strain (KM71FTEII), previously constructed in our laboratory (1) and producer of the thermostable beta-propeller phytase FTEII was used for all experiments. Two culture conditions that generate high (HEPP) and low (LEPP) extracellular phytase production (EPP) were selected, and three independent cultures for each culture condition were carried out in a 5 L bioreactor, as described elsewhere (2). During the induction step, expression levels of seven genes (*aox2*, *pep4*, *prb1*, *prc1*, *kex2*, *kar2*, and *fteII*) were measured by RT-qPCR, and each relative expression level to initial induction time was calculated by the $2^{\Delta\Delta Ct}$ method using two normalizer genes (*g6pd* and *ypt1*) with corrections for amplification efficiency and the dilution factor. For each culture, the EPP, percentage of phytase secretion, extracellular protease activity, cell growth, and methanol consumption were also determined in the induction step.

Results. The expression levels of *fteII* were similar for both tested culture conditions. However, since the methanol concentrations of the HEPP cultures were higher than those of the LEPP cultures, higher transcription levels of *fteII* were expected to occur in the HEPP cultures, compared to those of the LEPP cultures.

The expression levels of genes encoding for proteases (*pep4*, *prc1* and *prb1*) were significantly higher in the HEPP, compared to the LEPP cultures, for most of the induction step. Nevertheless, protease gene expression was not correlated with extracellular protease activity.

The expression levels of the *aox2* gene in the HEPP and LEPP cultures were not significantly different, despite the different methanol concentrations of the two tested culture conditions, and the fact that methanol is the inducer of the *aox2* gene. Although the *aox2* gene encodes for the enzyme in the first step of methanol metabolism, cell growth and methanol consumption were higher for the HEPP cultures than those of the LEPP cultures.

Expression levels of the *kex2* and *kar2* genes were significantly different for the two tested culture conditions, with the HEPP culture always being higher than the LEPP culture. The expression of *kex2* was correlated with the percentage of phytase secretion.

Conclusions. The EPP was not correlated with the transcription efficiency of the *fteII* gene. The expression of protease genes favors the EPP. The increase in EPP was due to an increase in EPP efficiency per cell. An improved protein folding process is likely involved in the EPP increase.

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