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Research article

Enhancing production of lipase MAS1 from marine *Streptomyces* sp. strain in *Pichia pastoris* by chaperones co-expression

Dongming Lan [a,1], Man Qu [a,1], Bo Yang [b], Yonghua Wang [a,*]

[a] College of Food Sciences and Engineering, South China University of Technology, Guangzhou 510640, PR China
[b] School of Bioscience and Bioengineering, South China University of Technology, Guangzhou 510006, PR China

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ABSTRACT

Background: A thermostable lipase MAS1 from marine *Streptomyces* sp. strain was considered as a potential biocatalyst for industrial application, but its production level was relatively low. Here, the effect of chaperones co-expression on the secretory expression of lipase MAS1 in *Pichia pastoris* was investigated.
Result: Co-expression of protein disulfide isomerase (PDI), HAC1 and immunoglobulin binding protein could increase the expression level of lipase MAS1, whereas co-expression of *Vitreoscilla* hemoglobin showed a negative effect to the lipase MAS1 production. Among them, PDI co-expression increased lipase MAS1 expression level by 1.7-fold compared to the control strain harboring only the MAS1 gene. Furthermore, optimizing production of lipase MAS1 with *Pichia pastoris* strain X-33/MAS1-PDI in a 30-L bioreactor were conducted. Lower induction temperature was found to have a benefit effect for lipase MAS1 production. Lipase activity at 24 and 22°C showed 1.7 and 2.1-fold to that at 30°C, respectively. Among the induction pH tested, the highest lipase activity was obtained at pH 6.0 with activity of 440 U/mL after 144 h fermentation.
Conclusion: Our work showed a good example for improving the production of recombinant enzymes in *Pichia pastoris* via chaperon co-expression and fermentation condition optimization.

1. Introduction

Enzymes play a key role in the "white biotechnology" due to their properties of high catalytic efficiency, substrate specificity, reaction under mild condition and environment-friendliness. Thus, they have found many applications in biotechnology industry, including detergents, cosmetics, foods, pharmaceuticals and flavor industry [1]. Currently, enzymes from microorganisms are attracting enormous attention because they are stable and can be obtained in bulk at low cost [2].

Although enzymes are great of value in industrial application, their yields are relative low via fermentation of original hosts. An alternative choice is production of recombinant enzymes in a heterologous host. Currently, several microorganism, including *Aspergillus oryzae*, *Aspergillus niger*, *Pichia pastoris (P. pastoris)*, *Saccharomyces cerevisiae* and *Escherichia coli*, have been developed for heterologous proteins production [3]. Among them, the methylotrophic yeast *P. pastoris* was considered as an excellent host for various proteins production, dues to its potential for high-level production, efficient secretion, high cell density growth and posttranslational modification. Several heterologous proteins, such as human serum albumin, have been reported to produce at grams per liter level by *P. pastoris* [4]. However, secretion level of some other proteins was in a relatively low level [5]. Therefore, strategies for elevating expression level of those proteins in *P. pastoris* are urgently needed.

Generally, over expression of recombinant proteins in eukaryotic cells, e.g., *P. pastoris*, might result in increasing production of unfold or misfolded proteins. The accumulation of those function-loss proteins in the endoplasmic reticulum (ER) could cause the activation of the unfolded protein response (UPR), which seriously affect the secretion and transportation of proteins [6]. Some cellular chaperones were found to have the functions for releasing the UPR stress to the cells. Protein disulfide isomerase (PDI) and immunoglobulin binding protein located in ER involve in formation of disulfide bond and refolding of protein [7], while HAC1 is a transcription factor for activating target genes coding for chaperones and foldases when UPR occurs [8]. Thus, co-expression of those chaperones with the target gene seems to be an effect strategy for enhancing the expression level of recombinant proteins [9,10].

* Corresponding author.
E-mail address: yonghw@scut.edu.cn (Y. Wang).
[1] These authors contributed equally to this work.
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Table 1
Primers used for construction of chaperon expression vectors.

Gene name	Gene length (bp)	Primer name	Primer sequences[a]
*mas*1	780	MAS1-F	5′-CGGAATTCGCCACGCCAGCTGCTGAGGCTACTT-3’
(H0B8D4)		MAS1-R	5′-CCGGTACCGCCAATCACAGAAGCACAGGTTGTA-3’
pdi	1554	PDI-F:	5′-AAACAACTAATTATTCGAAGGATCCAAACGATGCAATTCAACTGGAAT-3’
(EU 805807)		PDI-R:	5′-TAATTCGCGGCCGCCCTAGGGAATTCTTAAAGCTCGTCGTGAGCGTC-3’
*hac*1	996	HAC1-F	5′-CGCGGATCCATGCCCGTAGATTCTTCTC-3’
(XM 002489994.1)		HAC1-R	5′-CGGAATTCCTATTCCTGGAAGAATACAAAGTC-3’
bip	2037	Bip-F	5′-CGCGGATCCATGCTGTCGTTAAAACCATCTT-3’
(XM 002490982)		Bip-R	5′-ATTGCGGCCGCCTACAACTCATCATGATCATA-3’
vhb	441	Gene synthesis	
(AY 278220)			

[a] The restriction sites in the primers were underline.

Enhancement of microorganism hosts productivity can also be achieved by fermentation conditions optimization. Effects of carbon source, nitrogen source, dissolved oxygen (DO), induction temperature, pH and methanol concentration on the production of recombinant proteins have been extensively studied [11,12,13]. Among these fermentation factors, fermentation temperature and pH are key factors affecting the recombinant protein production and cell growth. Both cell death and target protein degradation could be decreased under low fermentation temperature [14]. Wang et al. [15] had reported that activity of recombinant polygalacturonate lyase in *P. pastoris* was found to be higher at low fermentation temperature than that at high fermentation temperature [15]. The pH value can affect the activity of recombinant proteins and cell growth by changing their environment charge. Charoenrat et al. [16] had reported that the proteolysis activity could be inhibited by pH control to increase the production of recombinant fungal endoglucanase in *P. pastoris* [16].

In our previous study, MAS1 lipase from marine *Streptomyces sp*. strain W007 was characterized as a thermostable enzyme which might have potentials for industrial application [17]. However, the yield of MAS1, constitutively expressed under control of glyceraldehyde-3-phosphate dehydrogenase gene promoter in *P. pastoris*, was found to be low. In here, to improve the expression level of MAS1 lipase in *P. pastoris,* the effect of co-expression of chaperones gene and optimization of fermentation conditions on lipase MAS1 production in *P. pastoris* in a 30-L bioreactor was investigated.

2. Material and methods

2.1. Strains, plasmids and materials

E. coli DH5α was used as cloning host. The plasmid pPICZαA and pPIC9K (Invitrogen, Carlsbad, CA, USA) were used as cloning vector. *P. pastoris* X-33 (Invitrogen, Carlsbad, CA, USA) strain was used for expression. 4-Nitrophenyl octanoate was purchased from Sigma-Aldrich (Shanghai, China). All other chemicals were of analytical grade.

2.2. Construction of expression strain

The *mas*1 gene was amplified using primers MAS1-F and MAS1-R (Table 1) with a previously constructed vector PGAPαA-MAS1 as template [17]. The PCR products were double digested with *Eco*RI and *Kpn*I, and ligated into the same sites in pPICZαA containing an AOX1 promoter to generate vector pPICZαA-MAS1. For construction of chaperones expression vectors, all the chaperone encoding genes were cloned into pPIC9K vector (Fig. 1b). PDI, HAC1 and BIP gene were obtained by PCR amplification using *P. pastoris GS115* genomic DNA as template. PCR product of *hac*1 and *bip* gene were digested by *BamH*I and *Eco*RI or *Not* I and ligated into the same site of pPIC9K, while PDI gene was cloned in the pPIC9K using In-Fusion™ cloning kit (Takara, Dalian, China) according to the instruction from the manufacturer. The *Vitreoscilla* hemoglobin (VHB) gene was synthesis by GENEWIZ biotechnology company (Shuzhou, China) and cloned into pPIC9Kvector. All the constructions were confirmed by fully sequencing.

P. pastoris X-33 was transformed with 10 μg of *Pme*I-linearized pPICZαA-MAS1 vector by electroporation to get X-33/MAS1. Transformants were selected by YPD medium plates (1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) dextrose, and 2% (w/v) agar) containing 100 μg mL^{-1} Zeocin. For construction of MAS1 and chaperon co-expression strain, X-33/MAS1 strain as the parent strain was transformed with linearized pPIC9K vector containing each chaperones gene by electroporation to get X-33/MAS1-PDI, X-33/MAS1-BIP, X-33/MAS1-HAC1 and X-33/MAS1-VHB strains. Transformants were selected by YPD medium plates (1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) dextrose, and 2% (w/v) agar) containing 100 μg mL^{-1} G418. Transformants containing inserts of chaperones gene were confirmed by colony PCR with their specific primers.

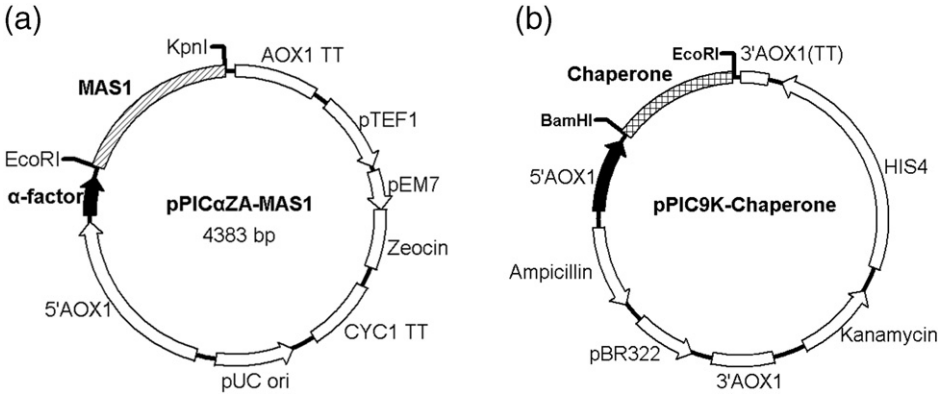

Fig. 1. Construction of expression vector. Physical maps of pPICαZA-MAS1 (a) and pPIC9K-Chaperone (b). The chaperones gene in pPIC9K included PDI, HACI, BIP and VGH.