Comparison of Alpha-Factor Preprosequence and a Classical Mammalian Signal Peptide for Secretion of Recombinant Xylanase \(xynB\) from Yeast \(Pichia pastoris\)

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The secretory efficiency of recombinant xylanase \(xynB\) from yeast \(Pichia pastoris\) between the \(\alpha\)-factor preprosequence and a classical mammalian signal peptide derived from bovine \(\beta\)-casein was compared. The results showed that although the bovine \(\beta\)-casein signal peptide could direct high-level secretion of recombinant xylanase, it was relatively less efficient than the \(\alpha\)-factor preprosequence. In contrast, the bovine \(\beta\)-casein signal peptide caused remarkably more recombinant xylanase trapped intracellularly. Real-time RT-PCR analysis indicated that the difference in the secretory level between the two signal sequences was not due to the difference in the transcriptional efficiency.

Keywords: Alpha-factor preprosequence, mammalian signal peptide, \(Pichia pastoris\), qPCR, copy number

The methylotrophic yeast \(Pichia pastoris\) has been engineered into a highly successful system for secreting a variety of recombinant proteins [6]. The \(Saccharomyces cerevisiae\) \(\alpha\)-factor preprosequence is the most widely used secretory signal in \(P. pastoris\). This sequence consists of a 19-amino-acid signal sequence (presequence) followed by a 66-amino-acid prosequence, which contains three potential N-linked glycosylation sites and a dibasic Kex2 endopeptidase processing site [8]. This signal sequence has been successfully used for directing a variety of heterologous proteins into culture medium. However, in many cases, the additional N-terminal Glu-Ala repeats were inefficiently processed. Moreover, hyperglycosylation could happen on the uncleaved prosequence [11]. Therefore, the \(\alpha\)-factor preprosequence is not an ideal leader sequence for obtaining correctly processed heterologous proteins, and searching for an alternative leader sequence would be required. The 15-amino-acid bovine \(\beta\)-casein signal peptide is one of the classical mammalian signal peptides. It directs secretion of \(\beta\)-casein protein into milk efficiently [7]. Moreover, this signal sequence has been successfully employed to direct high-level secretion of heterologous proteins into transgenic milk [13, 14]. We were curious to know whether this classical mammalian signal peptide is capable to direct secretion of heterologous proteins from yeast, and is correctly processed on the N-terminus, and become a promising alternative secretory signal of the \(\alpha\)-factor preprosequence. Therefore, in this study, the secretory efficiency of a thermostable xylanase \(xynB\) by \(\alpha\)-factor preprosequence and bovine \(\beta\)-casein signal peptide was compared based on single-copy-number integrants.

The xylanase gene \(xynB\), derived from \(Streptomyces olivaceoviridis\) A1 (GenBank Accession No. DQ465452.1), was synthesized by Sangon Biotech (Shanghai, China). To generate secretory expression vector pPICZ\(\alpha\)-XYNB, which contains the \(\alpha\)-factor preprosequence, the synthesized gene was cloned upstream of the His tag in the yeast expression vector pPICZ\(\alpha\)A (Invitrogen, CA, USA) (Fig. 1A). Similarly, to construct vector pPICZ-CSN2SP-XYNB, which contains the bovine \(\beta\)-casein signal peptide, the synthesized gene was first fused with bovine \(\beta\)-casein signal peptide, and then cloned upstream of the His tag in the yeast expression vector pPICZA (Invitrogen, CA, USA) (Fig. 1B). After electroporation of plamids into \(P. pastoris\) X33 competent cells, positive transformants were first screened on YPDS [1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) glucose, 1 M sorbitol, and 2% agar] supplied with 100 µg/ml Zeocin (Invitrogen, CA, USA) for 4 days [1], and then confirmed by PCR analysis. It has been recognized that multiple copies of transgenes may integrate after electroporation, and gene dosage has been proven to influence the expression levels of some proteins [4, 12, 15]. Therefore,
in order to draw a more reliable comparison result, we applied a quantitative real-time PCR (qPCR) method [15] to determine the copy number of transgenes in the positive transformants. Then, only single-copy-number integrants were selected for the comparison experiments. Positive transformants were cultured in flasks containing 20 ml of BMMY [1% (w/v) yeast extract, 2% (w/v) peptone, 1% (w/v) methanol, 1.34% YNB, 4 × 10^{-5}% biotin, 100 mM potassium phosphate (pH 6.0)] at 30°C while shaking at 275 rpm. During continuous culture of the cells, methanol was supplemented to a concentration of 1% (v/v) every 24 h to maintain the induction. The 200 ml aliquots of supernatant were collected every 24 h and used to measure the culture’s optical density at 600 nm and the enzymatic activity of the recombinant xylanase. Enzymatic activity was detected using the DNS method [2, 10]. As shown in Fig. 2A and 2B, both signal sequences were able to direct recombinant xylanase secreting into culture medium.