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Heterologous expression of a hydrophobin HFB1 and evaluation of its contribution to producing stable foam

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ABSTRACT

Hydrophobins are small secreted proteins belong to filamentous fungi. These proteins possess a unique ability to self-assemble at air/water interfaces. Hydrophobins have a broad range of biotechnological applications such as stabilizing emulsions and foams, immobilizing proteins on a surface, designing biosensors, affinity tag for protein purification, and drug delivery. We have successfully expressed HFB1 from *Trichoderma reesei* belonged to class II of hydrophobins in *Pichia pastoris*. The recombinant gene was under the control of the methanol-inducible AOX1 promoter (alcohol oxidase 1) in the pPICZAα vector. The amount of secreted HFB1 was increased in 90-h using methanol induction. The recombinant HFB1 was purified based on the presence of His-tag and foam formation. Furthermore, HFB1 was able to produce macro and micro stable air bubbles in the liquid due to the presence of hydrophobic patches on its surface. The liquid medium containing HFB1 becomes turbid after shaking, and then the stable bubbles are formed and remained for three weeks.

1. Introduction

Hydrophobins are small secreted proteins with a surface active, which are produced by filamentous fungi [1]. They consist of about 100 amino acids including 8 cysteine residues in conserved position, which can form 4 intracellular disulfide bonds [2]. Self-assembly in hydrophobic/hydrophilic (such as air/water) interfaces is one of the most important qualities of these proteins [3]. Their role in growth and development of fungi is of utmost importance. Hydrophobins help fungal hyphae to emerge from water by reducing the surface tension of water when secreted into the environment [4,5]. Moreover, they participate in coverage of cell wall fungi and subsequently lead to reverse hydrophobicity. These proteins facilitate appropriate gas exchange, aerial dispersal of spores and connection of hyphae to hydrophobic surfaces by aggregation on the surface of some structures such as fruiting bodies, spores, and hyphae, respectively [6,7]. Based on hydropathy profile, biophysical characteristics, and amino acid sequence similarity, hydrophobins are divided into two groups: class I and class II [8]. All hydrophobins can form amphipathic membranes. Membranes constructed by class I hydrophobins are resistant to dissolution and dissolved in formic acid or trifluoroacetic acid. But membranes created by class II hydrophobins are dissolved in 2% hot SDS or 60% ethanol [9].

Hydrophobins are useful in some branches of biotechnology such as stabilizing enzyme [10,11], biosensor and electrode construction [12], tissue engineering, drug delivery [13], foam stabilizer [14,15], coating techniques [16], and fusion tag for the purification of recombinant proteins [17]. There are many potential practical applications for this protein in technology; however, mass production with low cost is a major obstacle in this regard. Using a proper host such as *Pichia pastoris* could help surmount this problem. Protein production in *P. pastoris* is preferred among other hosts due to its wide range of post-translational modifications and production of secretory protein [18].

In this study, we have focused on the recombinant production of HFB1 from *Trichoderma reesei* belong to the class II of hydrophobins. The native form of HFB1 consists of 97 amino acids including 16 amino acids belong to the secretory signal sequence, 6 amino acids belong to the prepropeptide, and 75 amino acids belong to the main structural sequence. The final 7.5 KDa protein is delivered after elimination of the first two parts from N-terminal through intracellular processing. Moreover, we transformed hfb1 gene without a signal and prepropeptide sequences in *P. pastoris* and investigated

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the secretory expression of HFB1. We have also compared the efficiency of different methods on the yield of purification and finally presented a high-throughput method for purification of HFB1 with high purity and high concentration.

2. Materials and methods

2.1. Strains, vectors, and reagents

P. pastoris strain X-33, *Escherichia coli* strain DH5α was obtained from our laboratory. All restriction enzymes, T4 DNA ligase, and alkaline phosphatase were procured from Thermo Fisher Scientific, Inc. DNA extraction and plasmid purification kits were also supplied by Bioneer, Inc. The entire coding region of hfb1 gene was directly synthesized and cloned into the pUC57 vector by BioBasic, Inc. It was subsequently subcloned into the pPICZAα vector that was purchased from Iranian Gene Bank (Pasteur Institute of Iran), and Ni-NTA-sepharose resin was purchased from Invitrogen, Inc. Other chemicals were of analytical grade and obtained from Merck & Co., Inc.

2.2. Vector construction

The entire coding region of the hfb1 gene (278 bp) was directly synthesized and cloned into the pUC57 vector. Its coding sequence was synthesized according to Genbank sequence (accession no. Z68124) without sequences related to secretory signal and prepropeptide (Fig. 1). Two nucleotide fragments were inserted into the 5′ and 3′ ends of the hfb1 gene. These fragments were possessed restriction enzyme cleavage sites and added to both ends of the gene. The upstream segment contains the kex2 protease recognition sequence which was located after the Xho1 cleavage site (5′ end of the gene) and followed by sequencing related to alanine and glutamate. The downstream fragment includes a nucleotide sequence encoding the 6 His-tag previous the Not1 recognition site (3′ end of the gene). The pPICZAα vector which contains α-mating factor signal sequence was selected to produce secretory HFB1 protein. The gene of interest, after digestion with Xho1 and Not1 endonuclease, was ligated into Xho1/Not1 linearized pPICZAα plasmid. So that, hfb1 gene placed in a frame with α-factor secretion signal to ensure the efficient secretion of HFB1.

2.3. Pichia transformation

The pPICZAα-hfb1 plasmid was linearized with sac1 restriction enzyme and transformed into the X-33 strain of the *P. pastoris* by electroporation. Transformed plasmids were cultured on a YPDS agar medium containing Zeocin (100 mg/ml) for 5 days. 11 Zeocin-resistant clones were selected, and then integration of the hfb1 gene into the host's genome was approved by PCR.

2.4. Selection and expression of hfb1

6 clones of transformed cells containing the recombinant gene and a single colony of untransformed cells (X-33 strain) were grown in 5 ml of YPG medium at 30 °C with agitation at 250 rpm

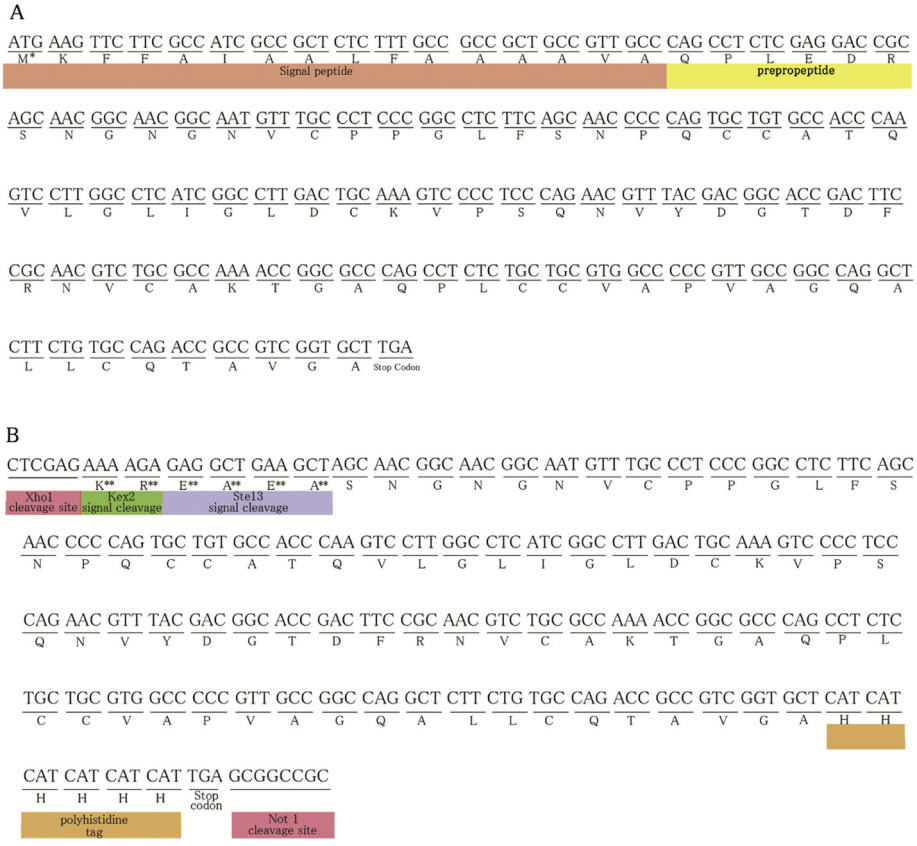

Fig. 1. General schemes of nucleotide and amino acid sequences. (A) The entire coding region of the hfb1 gene (accession no. Z68124) along with its related amino acid sequence. The HFB1 protein contains three parts: signal peptide, prepropeptide, and the hfb1 coding sequence. Initial methionine has been specified by a star (*). (B) Hfb1 nucleotide sequence expressed in *P. pastoris*. Two primary parts of the native gene have been omitted. Amino acids that have been specified by two stars (**) are cleaved by yeast endoprotease after expression.