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Soluble expression and purification of a functional harpin protein in *Escherichia coli*

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ABSTRACT

The use of harpins in practical agricultural applications may enhance plant growth and induce disease resistance. However, few investigations focused on the optimal expression and purification of harpin. In this work, harpin protein fused with a thioredoxin tag and a hexahistidine tag was expressed in *Escherichia coli* BL21 (DE3) cells as a soluble form under the induction of 0.5 mmol/L isopropyl β-D-1-thiogalactopyranoside. The purity of the recombinant harpin was greater than 90% after one-step nickel-nitrilotriacetic acid affinity chromatography. The yield of purified TRX-harpin protein reached 17.1 mg per 100 mL of cell culture. TRX-harpin is thermostable and could trigger the hypersensitive response effect in tobacco, with an efficient dose as low as 30 μg/mL. The root lengths of TRX-harpin treated tobacco and wheat plants were nearly 1.6-fold and 1.8-fold longer, respectively, than plants treated with the empty vector preparation. Thus, using a N-terminal TRX-tagged fusion is an economic way to produce bioactive harpin.

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1. Introduction

Harpins are proteins produced by gram-negative plant pathogenic bacteria. They share distinctive characteristics, heat stability, richness in Gly, no Cys, and few aromatic amino acids [1,2], which are distinct from those of other bacterial proteins. Once applied to plants or expressed in transgenic plants, harpins induce reactive oxygen species (ROS), and hydrogen peroxide (H_2O_2) in particular, as well as the hypersensitive response (HR) effect [3,4]. The HR effect is a highly localized defense response that is characterized by the rapid death of plant cells at the site of pathogen invasion [5]. These defense responses induced by harpins consist of a complicated defense signal transduction network, which utilizes mutual coordination to enhance disease resistance in plants. The exogenous application of harpins can induce systemic acquired resistance and enhance the defense response against diverse pathogens and insects, as well as stimulating plant growth [6–10]. Spraying with harpin increases the productivity and biochemical properties of green tea [11], and harpin treatments induce drought tolerance in Arabidopsis [12]. Overall, harpins have been recognized as multifunctional elicitors in plants. Foliar treatments with elicitor molecules are an attractive alternative to conventional ecologically unsafe pesticides for the control of plant diseases. Furthermore, harpins accelerate plant growth and development by increasing root and shoot biomasses, which are desired agricultural effects [13–15]. Because of its effects on plants, harpins hold promise for practical agricultural use to enhance plant growth and induce disease resistance. Therefore, it is necessary to mass-produce harpins at a low cost.

Traditionally, harpins were obtained by plant pathogenic bacteria, like *Pseudomonas*, *Erwinia*, *Xanthomonas* and *Ralstonia*. The most common forms of harpins were secreted upon interactions with plants and subsequently delivered into plant apoplasts through the bacterial type III secretion system [16,17]. These methods prevent harpin' applications in agriculture because of the high cost of production. With the development of genetic engineering technology, harpin gene sequences were identified and cloned for the first time in 1992 [2]. Then, harpins were genetically engineered. Briefly, harpin gene sequences were cloned into multi-copy plasmid vectors to overproduce these proteins [18–21]. Several other harpins have been characterized from various Gram-negative plant pathogenic bacteria: HrpN and HrpW of *Erwinia* spp.; HrpZ, HrpW, HopPtoP and HopPmaHpto of *Pseudomonas syringae*; PopA1 of *Ralstonia solanacearum*; and HpaG and its orthologs of *Xanthomonas campestris*, like XopA [1,6,22–26]. There were even several reports of multifunctional characterizations of these proteins, but few

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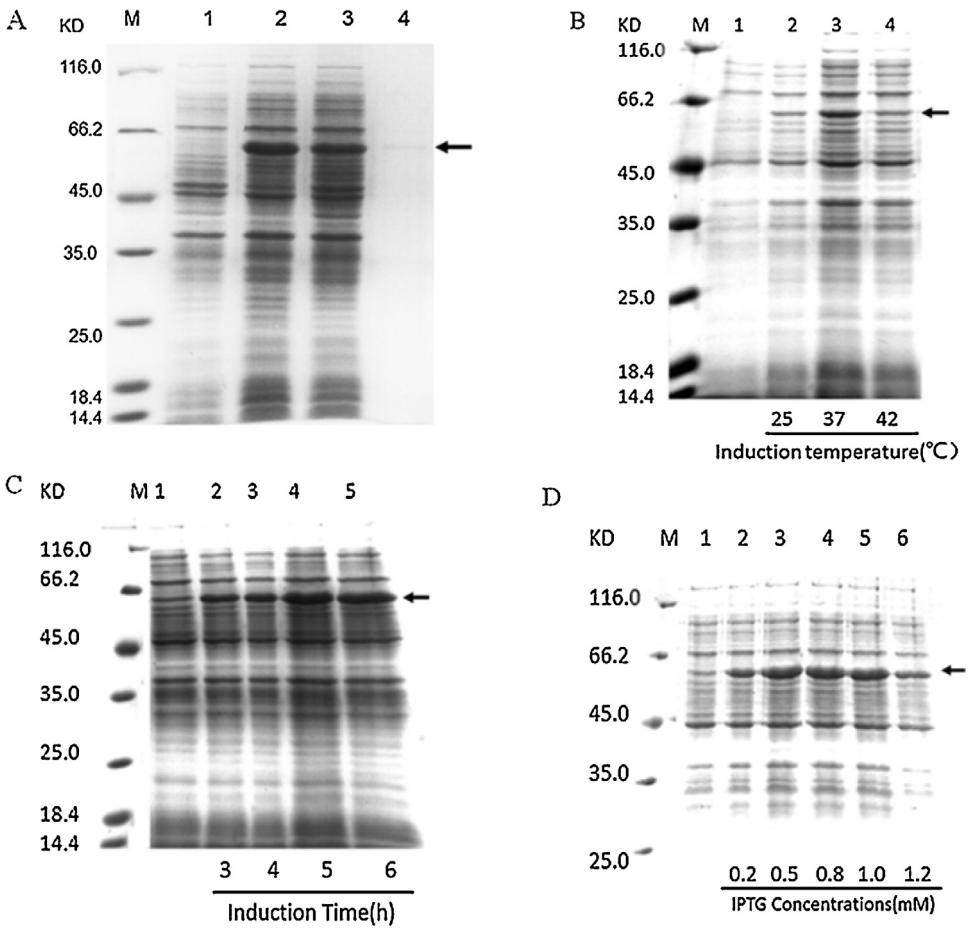

Fig. 1. Over-expression of TRX-harpin in *E. coli* BL21 (DE3). A, SDS-PAGE analysis. Lane M, protein molecular weight standers; Lane 1–2, non-induced/induced lysates of TRX-harpin; Lane 3, soluble fraction; Lane 4, insoluble fraction. B and C, Effect of induction temperature and time on the level of expression of TRX-harpin in BL21 (DE3). D, Effect of ITPG concentration on the level of expression of TRX-harpin in *E. coli* BL21 (DE3). The concentration of IPTG was varied as follows: 0.2, 0.5, 0.8 and 1.0 mM, respectively. Expression of the protein was carried out at 37 °C for 5 h. Arrows indicate the positions of the target proteins.

investigations focused on the optimal expression and purification of harpins [17,27]. The latter is needed, not only for exploring their biological functions, but also for improving their industrial applications [25,28–30].

Because harpins are promising molecular elicitors with practical agricultural applications that could enhance plant growth and induce disease resistance, in the present study, we developed an efficient system for producing large quantities of soluble recombinant harpin using a one-step purification process. HR and plant promotion assays were used to test the activity level of the recombinant harpin.

2. Materials and methods

2.1. Materials

The restriction endonucleases *Eco*RI and *Xho*I (TaKaRa, Japan), as well as T4DNA ligase (TaKaRa) were used according to the manufacturer's specifications. *Escherichia coli* DH5α (maintained in our laboratory) was used for subcloning and plasmid amplification. *E. coli* BL21 (DE3) (maintained in our laboratory) was used as the expression host. The concentration of total proteins was determined using a bicinchoninic acid (BCA) Protein Assay Reagent Kit that was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Ampicillin and isopropyl ß-D-1-thiogalactoside (IPTG) were purchased from Dingguo (Beijing, China). A nickel-nitrilotriacetic acid (Ni-NTA) column was purchased from Amersham Biosciences (Uppsala, Sweden). The anti-His-tag antibody was purchased from Epitomics (Burlingame, CA, USA). Seeds of the tobacco were provided by Dr. Xiaofang Li (ECNU, Shanghai, China) and the wheat was purchased from a local market. The other reagents were all of analytical grade and used as received.

2.2. Plasmid construction

The mature harpin-coding sequence of HRP (GeneBank Accession Number:AY999003.1) was optimized to further improve the expression level. Several codons of the original *HRP* gene were changed according to *E. coli*'s favorite codon usage from the codon usage database (Web site: http://www.kazusa.or.jp/codon/countcodon.html). The optimized gene sequence, consisting of the codons for 403 amino acids, was synthesized with an *Eco*RI restriction site incorporated at the 5′-end and an *Xho*I restriction site at the 3′-end. The gene sequences were synthesized by Generay (Shanghai, China). Afterward, the optimized *HRP* gene sequence was ligated into the pET32a (+) vector. The recombinant vector pET32a-HRP was transformed into *E. coli* DH5α cells using the calcium ion method and plated on ampicillin-containing agar plates. Single colonies were screened for successful ligation events by restriction digestion and DNA sequencing (Life Technologies, Carlsbad, CA, USA).

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