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Cell disruption enhanced the pure EGFP recovery from an EGFP-intein-surface protein production system in recombinant *E. coli*

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ABSTRACT

In this study, three approaches for protein production were devised to enhance the efficiencies in yielding high purity protein via surface display system. A plasmid carrying enhanced green fluorescent protein (EGFP), an intein (INT) and ice nucleation protein (INP) was constructed to produce EGFP via surface display in *Escherichia coli*. To obtain high purity of the produced EGFP, several procedures, including osmotic shock, surfactant addition and cell disruption were employed. Among these approaches, the cell disruption method gave the highest EGFP purity by simply conducting several centrifugations. An EGFP yield of 63 mg/L with 97% purity was obtained. The result demonstrated that pure EGFP can be harvested only through centrifugation; no complicated processes or expensive equipment are required. This approach shows potential for the production of pure recombinant proteins in scale-up processes for biotechnological, academic and industrial uses.

1. Introduction

Functional proteins are widely needed in the fields of food, chemistry and medicine; however, the production of pure proteins is often costly and laborious due to the complicated processes required. Chromatography with an affinity tag is generally applied for protein purification. In the literature, an affinity purification procedure specifically designed for the tagging of the fusion proteins has been established [1–3]. For example, proteins with polyhistidine tags (His-tag) were harvested via immobilized metal affinity chromatography (IMAC) [1,4,5]. However, the major disadvantages of this method are low production, high time consumption, expensive equipment and difficulty in scale-up [6].

An approach that generates the target protein from the fusion protein without the use of specific proteases has been developed. This approach uses an autocatalytic protein called intein (INT) that contains self-cleavage elements. Owing to this property, many protein engineering applications are taking advantage of intein in the protein production process [6–9]. By fusing to the N- or C-terminus of the target protein, intein acts as a useful tool in the protein purification procedure. In this study, Ssp DnaB intein from IMPAC-TWIN system (New England Biolabs, Beverly, MA) was chosen because of its' cleavage reaction was inducible via only pH and temperature shifts.

Ice nucleation protein (INP), an outer membrane protein is found in several plant pathogenic bacteria and enables the formation of ice on the surface of the bacteria [10,11]. INP is considered to be a good carrier protein due to its characteristics such as capable of expressing larger foreign proteins to more than 60 kDa and possessing the modifiable length which might avoid the steric hindrances of the displayed protein [12]. In the literature, the target proteins were fused to the C-terminus of INP and the engineered bacteria were found to exhibit the surface-localized activities of the target proteins [13–16].

In a previous study, the INP-INT system was developed to produce a model protein, EGFP, via recombinant *E. coli* cultivation, induction and centrifugation [17]. However, there are still issues arising from this process. First, it took a long time (about 5 days) to achieve the cleavage process. Second, the purity of the harvested EGFP (about 50%) was not as high as expected. It was found that

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cell autolysis during the long cleavage time and a high pH condition might contribute to the low protein purity. The harvested protein might inevitably be contaminated by the lysed cells in the process. In this study, methods, including osmotic shock, surfactant addition and cell disruption, were introduced to facilitate the INP-INT system process to increase EGFP purity and productivity. Surfactants, including Triton X-100 and Tween 20, were used. In addition, magnesium ion content in the low osmotic strength solution in the osmotic shock method was studied. Furthermore, a French Press was employed to disrupt the cell in order to facilitate EGFP production. A comparison of the protein production in the literature with the methods developed in this study was also carried out and discussed.

2. Materials and methods

2.1. Bacterial strains and plasmids

Construction of plasmid pINP-INT-EGFP was carried out according to that described by Wu et al. [17]. In brief, ice nucleation protein (INP) gene, Ssp DnaB intein (INT) and EGFP were obtained from plasmids pETInaXNC1, pTWIN1 (New England Biolabs, Beverly, MA) and pEGFP (Genetech, Takarabio, USA), respectively. A PCR product of INT-EGFP fragment was obtained via recombinant PCR from pTWIN1 and pEGFP, and then constructed in plasmid pETInaXNC1 to yield plasmid pINP-INT-EGFP as shown in Fig. 1. The *Escherichia coli* strain DH1 (DE3) carrying recombinant plasmid was

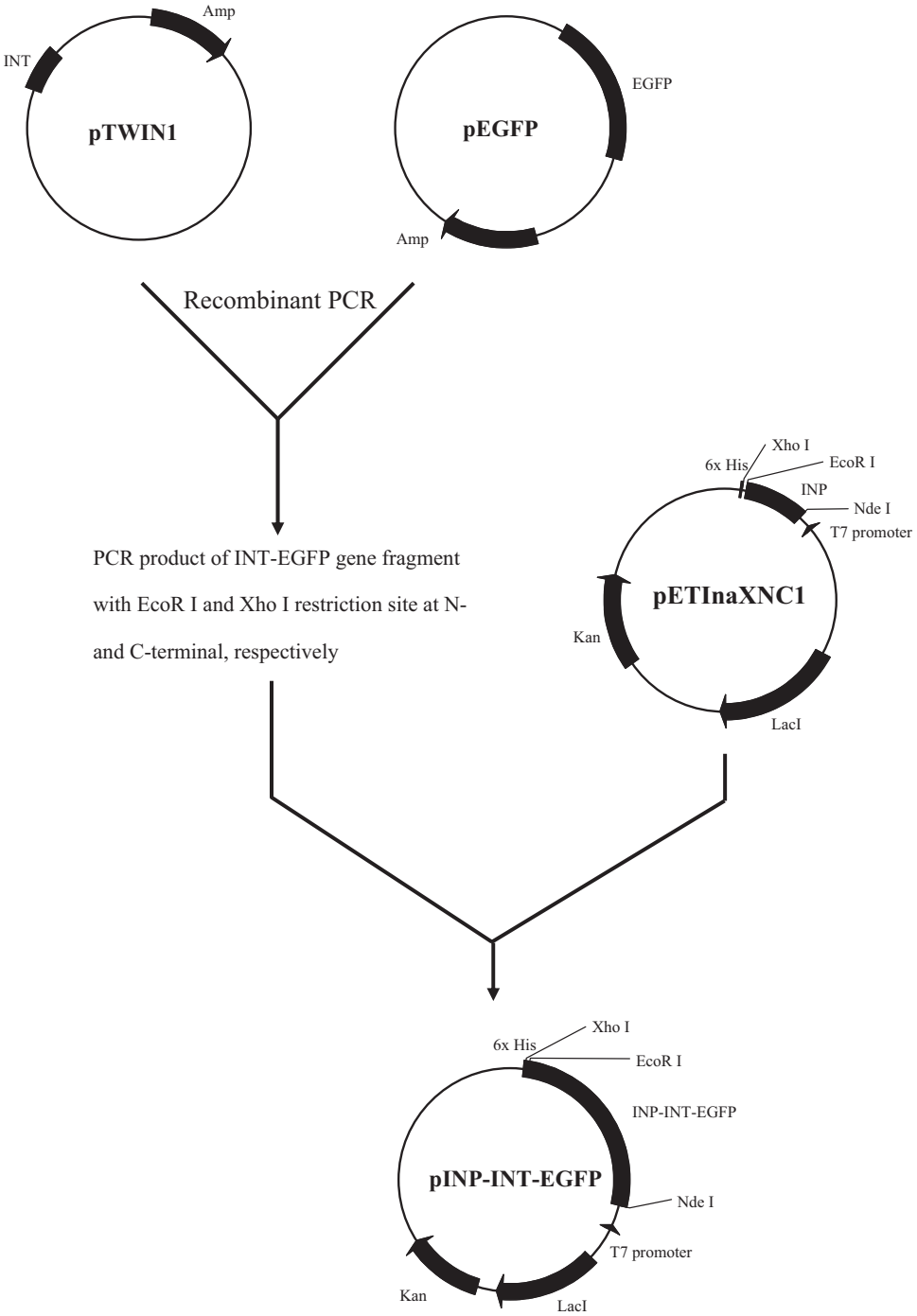

Fig. 1. Schematic graph displaying the construction of plasmid pINP-INT-EGFP.