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The Efficient Optimization of a Protein Expression by Design of Experiment

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Abstract

In terms of the prokaryotic expression system *Escherichia coli*, it is well-known that incubation temperature, OD_{600} value, isopropyl thiogalactoside concentration and time of induction are general factors which can directly affect the final protein's expression. The traditional method for this optimization is to study these factors which may influence the protein expression separately, thus, the interactions between factors are ignored. Design of experiment is a time-saving and cost-effective methodology for increasing the productivity and improving the quality of a product. In order to verify if design of experiment is suitable for the optimization of a protein expression and also to reveal if there is any interaction between the factors that have a significant influence on the protein's expression, the optimization of expression for the protein *Bmlebocin3* was carried out using the design of experiment concept. Bmlebocin3 is an antimicrobial peptide in the silkworm, *Bombyx mori*. It has a unique precursor form which was suitable for this investigation. In this study, the *Bmlebocin3* gene was cloned and expressed in *Escherichia coli*, and then this expression was optimized by design of experiment. The results revealed that through the use of design of experiment, in addition to quantifying the separate effects of the OD_{600} value and the time of induction, it demonstrated that, their interaction can significantly affect the expression of *Bmlebocin3*. Furthermore, the best condition for the *Bmlebocin3*'s expression was identified through fewer experiments. This proved that design of experiment can be used as an efficient way to optimize the protein expression.

Keywords: DoE; Vector Construction; Optimization; Protein Expression; *Bmlebocin3*

1 Introduction

Heterologous expression of a protein is a necessary technology in molecular biology. It is widely used to obtain a protein in a large scale where it is difficult to isolate it directly from original

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organisms. *Escherichia coli*[1] (*E.coli*), yeast and various kinds of cells are available as the host for recombinant protein expressions [1–3]. No matter which expression system has been chosen, the optimization of the expression is essential after the target protein has been validly expressed.

Optimization is the procedure used to find the best condition for the target protein expression so that the maximum production can be obtained with the lowest cost in the shortest time. In terms of the prokaryotic expression system *E.coli*, many factors which have a potential influence on the protein's expression are usually considered during optimization; these include, codon usage, copies and stability of the expression vector plasmids, structure and stability of mRNA, the growth condition of the host such as the pH of the culture medium, agitation, oxygenation, and the carbon source and so on [4, 5]. In addition, it is verified that incubation temperature, the OD_{600} value when induction begins, the isopropyl thiogalactoside (IPTG) concentration and the time of induction can also directly affect the production of a recombinant protein's expression, so they are general factors to be considered in the optimization. The traditional method for this optimization is to set each factor as a variable in turn whilst fixing the others, this means the factors are investigated one by one, and then are combined to define the best condition [6]. This can be both complicated and time-consuming. Furthermore and most importantly, by using this method, the effects of interactions between the factors ignored.

Design of Experiment (DoE) is a scientific approach to find a time-saving and cost-effective way to increase productivity and to improve product quality in the areas of physical, materials, social sciences and agricultural engineering [7–11]. It is an efficient method that can be used to change one or more process factors so that the corresponding yields and conclusions are determined. In this study, DoE was utilized to determine if it is suitable for the optimization of a recombinant protein expression and also to reveal if there is any interaction between factors having a significant influence on this expression. The protein chosen in this study was a precursor lebocin3, which is a kind of antimicrobial peptide (AMP) from silkworm [12]. Precursor lebocin3 is not a functional protein so it is very suitable for an investigation into the optimization of a recombinant protein expression. In this study, *Bmlebocin3* precursor gene was cloned and expressed in *E.coli* strain BL21, and then DoE was implemented to optimize this expression.

2 Materials and Methods

2.1 Gene Cloning

The primers for the *Bmlebocin3* precursor gene (GenBank accession no. AB003035) were designed as follows, forward primer, 5'- CATG**CCATGG**GCCAGAGGTTCATCCAGCCGACC-3' and reverse primer, 5'- TT**GCGGCCGC**TTAATGATGATGATGATGATGTTCTTGGAAAATGTCCCTCG -3' (Invitrogen), *Nco*I and *Not*I restriction enzyme sites (in bold) were introduced to facilitate vector construction, the relative sequence of six His tag (underlined) was placed ahead of the stop codon for western blot analysis.

The template for the PCR reaction was the fat body cDNA of silkworms (Dazao) after injected induction by DH5a. The PCR reaction condition was as follows: preheating to 94°C for 5 min, 30

[1] *Abbreviation used*: *E.coli*, *Escherichia coli*; OD, optical density; DoE, design of experiment; LB, Luria-Bertani; IPTG, isopropyl ß-D-1-thiogalactoside; PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline.