Expression of Antibacterial Cationic Peptides from Methylotrophic Yeast, *Pichia pastoris*

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Abstract

Antimicrobial cationic peptides have attracted increasing research and clinical interest as a natural antibiotics due to their broad spectrum of antimicrobial activites and the rapid development of multidrug-resistant pathogenic microorganisms.

In this study, first, we synthesized artificial fusion partner and cationic peptide genes (lactoferricin, magainin, protegrin-1, and indolicidin). Second, we constructed recombinant expression vectors and then transformed *Pichia pastoris*. Finally, expressed cationic peptides were purified and tested for their antimicrobial activites. Antimicrobial activity has been tested upon the appearance of clearing zone on the plate with the lawn of gram negative *E.coli* XL-I blue and garm positive *Staphylococcus aureus*. Protegrin-1 and Indolicidin have apparant activity of cationic peotides. This fusion technique may lead to a general and suitable tool for production of pure antimicrobial cationic peptides in *Pichia pastoris*.

Key Words : cationic peptide, fusion partner, *Pichia pastoris*

Introduction

Antimictobial cationic peptides are important components of the innate defenses against microbes in all species of life such as insect, plants, amphibians, and mammals[1]. These peptides have various structures and functions including antibacterial, antifungal, antiviral, and antiendotoxin activities[2]. Also, the emerging microbes having resistance to conventionally used antibiotics has triggered considerable interest in the structure-function relationship studies in short antimicrobial peptides in recent years[3]. With this reason, We developed a recombinant *Pichia pastoris* producing several antimicrobial peptides (lactoferricin, magainin, protegrin-1, and indolicidin) through genetic engineering. However, this expression system has

limitations- antimicrobial activity against the host bacterium and proteolysis during recombinant peptides production. To overcome this problems, we introduced acidic fusion partner, modified promagainin from the skin of *Xenopus* laevis as described by Lee et al[4]. Fusion partner is expected to neutralize the basic cationic peptides.

In this study, we have focused on the expression of cationic peptides fused to an acidic fusion partner in *Pichia pastoris*.

Materials and Methods

1) Strains and plasmids

E. coli Top10 F: plasmid propagation

Pichia pastoris GS115: host for fused peptides expression

pBSII(+): cloning vector, pPIC9K: expression vector

2) Construction of synthetic gene assembly

Four cationic peptides and modified promagainin (MPM) fused cationic peptides (CPs) were chemically synthesized

3) Confirmation of the expression vectors by direct PCR

4) Transformation into *Pichia pastoris* GS115

5) Screening of *Pichia pastoris* transformants

6) Production, purification and antimicrobial activity of cationic peptides

Results and discussion

1) We designed synthetic coding genes for CPs and MPM based on the peptide sequence, using *Pichia pastoris* codon preference, and these CPs and MPM were ligated to pBS II vector. All of the final constructs were identified by DNA sequencing.

2) In many propeptides, proregion is required to inactive otherwise toxic propetide to its host. CP may toxic to *Pichia* host. So to neutralize the basic cationic nature of CPs, acidic anionic proregion dereived from *Xenopus laevis* introduced in this study[5].

3) CPs or MPM-CPs fragments were cleaved from pBS II and then ligated to the multi-cloning site of pPIC9K expression vector. To confirm the insertion of CPs and MPM in pPIC9K, colony PCR was carried out.

4) Electrotransformed cells were screened for multicopy transformants using G418 selection method. Nine colonies were appeared at 4mg/ml of

G418 concentration. Transformants were analyzed by direct PCR using *Pichia* genomic DNA as a template and expression of mRNA was confirmed by RT-PCR using *Pichia* cDNA as a template.

5) The fusion proteins were expressed in crude supernatant and yeast extract and subjected to reverse-phase HPLC[6] and the collected fraction was cleaved by CNBr.

6) Electrophoretic analysis of recombiant CPs on tricine SDS-PAGE[7] was carried out. Only protegrin-1 and indolicidin were detected.

7) The purified recombinant protegrin-1 and indolicidin were tested for its antimicrobial activity against gram negative bacteria *E.coli XL-I blue* and gram positive *Staphylococcus aureus* by plate assay. Only CPs purified from yeast extract had an antimicrobial activity.

Many efforts have to be put to optimize the present *Pichia* system for the synthesis of cationic peptides. In near future, the expression system in *Pichia pastoris* may provide a suitable tool for production of pure antimicrobial cationic peptides.

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