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Expression and Secretion of Active Insect Defensin in *Pichia Pastoris*

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Antimicrobial peptides are a part of innate immune systems widely distributed in mammals, plants, fish, amphibians and insects, and play a key role in the early host defense response against infections of pathogenic microorganisms. Many insects have long been known to produce inducible antibacterial peptides in response to invading pathogenic bacteria to kill them. Defensin A derived from *Phormia terranovae*, one of insect defensin family, which has antibacterial activity against Gram-positive bacteria, is composed of 40 amino acids and contains 6 cysteine residues.

Recently, these antimicrobial peptides have drawn much interest as the potential of therapeutic agents to overcome the side effect of commercial antibiotics like the appearance of antibiotics-resistant pathogenic bacteria and the accumulation of antibiotics in the human body. The study of antimicrobial peptide on the elucidation of the mode of action, structure-function relationship or the evaluation of their applicability needs relatively large amount of peptides. However, the limit of immunized host and low yield of peptide purification necessitates an efficient recombinant expression system. So far, several antibacterial peptides has popularly been produced as fusion proteins in *E. coli,* but this method needs additional purification step to obtain biologically active peptides.

We carried out the expression and secretion of defensin A in yeast to establish a model system for the secretory production of biologically active antibacterial peptide. Defensin A gene was chemically synthesized with 6 oligomers and assembled *in vitro*. A recombinant plasmid containing the promoter of alcohol oxidase gene (*AOX1*), the preprosequence of mating factor 1 (MF1) and defensin gene were constructed and integrated into chromosomal DNA of *Pichia pastoris* through homologous recombination.

Transformed yeast cells were selected on the histidine-deficient minimal agar plates and then tested for G-418 resistance in a doe-dependent way. Four yeast clones were re-selected for further analysis. Southern blot and RT-PCR analysis revealed that defensin gene was stably maintained and transcribed in these cells. The secretion of active defensin from these cells were confirmed by growth inhibition zone assay using *Micrococcus luteus* IAM1056 as a test organism. The active defensin was purified to homogeneity through ammonium sulfate precipitation, SP-Sepharose column chromatography and reverse phase HPLC and it's phyico-chemical properties were investigated. The purified defensin was correctly processed in *P. pastoris* and had molecular weight of 4 kDa consistent with that deduced from nucleotide sequence.

The defensin purified from yeast supernatant also showed antibacterial activity against *St. aureus, L. monocytogenes, B. coagulans, B. subtilis, L. plantarum* and *P. acidilactici*. And it showed high heat-stability and more than 87% of residual activity was retained for up to boiling 30 min. Furthermore, the treatment of buffer solutions for 2 h, with pH range from 2.0 to 12.0 had no effect on the activity. The defensin was resistant to the treatment with amylases, lipase and cellulase but sensitive to proteases

such as protease K, subtilisin, pronase, protease IV, protease IX and trypsin except pepsin. The defensin showed the growth inhibitory activity against *M. luteus* in liquid culture with LC_{50} value of about 7 /. The study on culture characteristics is now in progress to improve defensin productivity.

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