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学 位 論 文 内 容 の 要 旨
Abstract of Doctoral Dissertation

博士の専攻分野の名称　博士（生命科学）　氏 名
Degree requested　Doctor of Life Science　Applicant name　MD. RUHUL KUDDUS

学 位 論 文 題 名
Title of Doctoral Dissertation

Construction of the over-expression system of cysteine-rich plant antimicrobial peptide snakin-1
(植物由来システインリッチ抗菌ペプチド snakin-1 の大量発現系の構築)

Antimicrobial peptides (AMPs), also known as host defense peptides, are characterized as small (<100 residues) cationic peptides widely distributed in nature. These multifunctional peptides are thought to kill invading pathogens by formation of membrane pores, resulting in cell leakage and ultimately, cell death. It is generally assumed that three main mechanisms could account for peptide permeation of the membrane of the target cell, including “barrel-stave model”, “carpet model” and “toroidal-pore model”. Due to their natural antimicrobial properties and a low propensity for the development of bacterial resistance, AMPs have rapidly captured attention as a rich source of lead compounds for the discovery of novel peptide antibiotics. For this reason, study in this field has been emphasized on screening novel AMPs.

Snakin-1 (SN-1) is a cysteine-rich plant antimicrobial peptide and the first purified member of the snakin family. SN-1 shows potent activity against a wide range of microorganisms, and thus has great biotechnological potential as an antimicrobial agent. To accomplish extensive structural and functional research, high-level expression of snakin peptide in the functionally active form is necessary. Therefore, the aim of the current study was to construct efficient overexpression system in order to obtain a large amount of biologically active SN-1 peptide.

In chapter 1 of this thesis, I have utilized the *Escherichia coli* expression system for enhanced production of recombinant SN-1. Since, *E. coli* is easy to handle, grows rapidly, and used to produce more than 80% recombinant AMPs. However, the direct expression of AMPs in *E. coli* poses some difficulties, such as their toxicity towards the expression host as well as susceptibility to protease. The expression of AMPs in the form of inclusion bodies are shown to solve these problems. But, it is not easy to control the inclusion body formation of target peptides directly during the recombinant expression of AMPs.

Therefore, in chapter 1, I applied the coexpression method to produce potato SN-1 through the enhanced accumulation of inclusion bodies in *E. coli* cell cytoplasm. The yield of SN-1 by the coexpression method using aggregation-prone partner protein was better than that by direct expression in *E. coli* cells. After HPLC purification, I was succeeded to obtain several milligrams of functionally active SN-1. MALDI-TOF MS and NMR analysis indicated that the recombinant SN-1 produced by coexpression method had the same folding when compared to native potato SN-1. In addition, I have determined its antimicrobial activity by minimum bactericidal concentration (MBC) measurements.

In chapter 2 of this thesis, I successfully constructed an overexpression system of SN-1 using methylotrophic yeast *Pichia pastoris* GS115. This yeast was selected because *P. pastoris* cells under the control of a methanol-induced AOX1 promoter can produce large amounts of functionally active cysteine-rich AMPs that are secreted directly into the culture media. I obtained large amount of pure recombinant SN-1 (Yield = 40 mg/1L) from a fed-batch fermentation culture after purification with a cation exchange column followed by RP-HPLC. Moreover, I have confirmed the identity and disulfide connectivity of recombinant SN-1 by MALDI-TOF MS, CD and ^{1}H NMR experiments. Additionally, I examined the membrane permeability of recombinant SN-1 using *E. coli* ML35. The assay results showed that SN-1 exhibited bactericidal activity through the membrane disruption of target cells.

In this thesis, I present the efficient expression system of plant AMP SN-1 using both prokaryotic and eukaryotic expression host. Therefore, I hope this study can serve as a suitable expression scheme for the large-scale production of disulfide-rich AMPs necessary for both research and application purposes.