

B-5. Cloning and expression of *Actinobacillus actinomycetemcomitans* CDT

Eun-Sun Lee*, Sun-young Ko, Jun-Ho Oh, Young-bum Ahn,
Hyung-Seop Kim, Moon-Taek Chang

Department of Periodontology and Institute of Oral Bioscience,
School of Dentistry, Chonbuk National University

Introduction

Cytolethal distending toxin (CDT) constitute a family of genetically related bacterial protein toxins able to stop the proliferation of numerous cell lines. This effect is due to their ability to trigger in target cells a signaling pathway that normally prevents the transition between the G2 and the M phase of the cell cycle.

The CDT of *A. actinomycetemcomitans* are determined by a cluster of three adjacent gene (*cdtA*, *cdtB*, *cdtC*) encoding proteins whose respective role is not yet fully elucidated

Objects

The aim of this study is to find the *A. actinomycetemcomitans* CDT can be reconstituted in vitro *CdtA*, *CdtC*, and purified the reconstituted CDT and to investigate the function of CDT in the bacterial pathogenesis.

Methods

A. actinomycetemcomitans (ATCC29522) was used for cloning the *cdtA*, *cdtC* genes.

The genomic DNA of *A. actinomycetemcomitans* was isolated using the genomic DNA extraction kit and used as template to yield *cdtA*, *cdtC* genes by PCR. The amplified *cdtA*, *cdtC* genes were cloned into T-vector. The cloned *cdtA*, *cdtC* gene was then subcloned to pQE30 expression vector, yielding pQE30-*cdtA*, pQE30-*cdtC*.

The bacterial host was transformed with pQE30-*cdtA*, -*cdtC* and the induced to express CDT protein

Results

The gene for CDT protein of A.a. was cloned and its integrity was confirmed by nucleotide sequencing. The cloned *cdtA*, *cdtC* was subcloned into pQE expression vector. Diverse conditons were tested to opimize the expression and purification of functional CDT in E. coli. The procedure for the purification of functionally active CDT pretein is in progress.

In consideration of signification of CDT in cell cycle active recombinant CDT protein will serve to investigate the pathological process of A.a. in periodontal disease.