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International Centre for Genetic Engineering and Biotechnology
United Nations Industrial Development Organization

Collaborative Research Programme

TERMINAL EVALUATION REPORT

UNIDO contract # 91/053

ICGEB ref. #: CRP/CHN90-03

Project initiation: 1991.9

Project termination: 1995.9

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TERMINAL EVALUATION REPORT

Part 1

Title of Project	
A novel efficient and powerful method for site-specific mutagenesis	
Keywords: site-specific mutagenesis, multi-point mutagenesis, subtilisin E, mutant library, screening by expresion	
UNIDO contract # 91/053	**ICGEB ref. #:** CRP/ CHN90-03
Project initiation: 1991.9	**Project termination:** 1995.9
Principal Investigator's name: Qisong Wang, Yi Xie	
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Abstract:	

We have established a new, simple and efficient method for site-specific mutagenesis. The principle of which is still based on oligonucleotide-directed site-specific mutagenesis.The method enables to obtain large amount of mutations with various mutant-site combinations in only one reaction by means of annealing dozens of mutagenic primers to a target sequence simultaneously. Since the resulting mutants are screened preliminarily by expression, no hybridization and sequencing is need for thosemutants without desired phenotypes. Compared with other methods,the method has the advantages of high-efficiency, simplicity, time-saving and controllability. It could be used to change the characterization of the enzyme, for instance, to makethe enzyme more thermostable,more stable at higher or lower pH,more active etc.

To set up this method, we have selected the subtilisin E gene as our target. according to the published results. 15 mutagenic primers for subtilisin E have been designed to check if the method is good or not. The results which are obtained testify the method feasible.

Part 2

OBJECTIVES/METHODOLOGY
(proposed at the time of the submission of the research proposal)

1. To establish a powerful site-specific mutagenesis method which enables large anount of mutations to occur in only one reaction.
2. To construct a mutant library of subtilisin E for about 15 mutagenic sites
3. To obtain several mutants with desired properties.
4. To publish a paper about the new method

RESULTS
(compare against the set objectives)

1.We have established a simple and efficient method for multi-point site-specific mutagenesis. The procedure includes:annealing to a single-stranded uracil-containing DNA template with fifteen oligonucleotide fragments at a time, in vitro synthesis and ligation, transforming JM109 of E.coli, cloning the mutant gene mixtures into the vector which can express heterologous gene,screening the mutant genes by phenotype selection.

Compared with other methods,the method has the advantages of high-efficiency, simplicity, time-saving and controllability.

2.We have contructed a mutant library of subtilisin E containing various mutantions for fifteen mutagenic sites. The random library has been selectively screened to be reliable by dot-blot hybridization and DNA sequence analysis.

3.We have obtained five mutants exhibiting more thermostable, more stable at higher pH or/and resistant to oxidation. They all have been sequenced out and found to differ from wild-type subtilisin E. Three by one amino acid(Met222Ala,Try104Phe,Met222Cys),one by three amino acids(Asn76Asp/Asn109Ser/Ile205Cys),the other by four amino acids(Asn76Asp/Asn109Ser/Ile205Cys/Met222Cys).

4.Now we have no less than three articles to be published:

1)construction of site-mutant subtilisin E pool with a set of oligonucleotides. Science in China(series B)(press in chinese and English)

2)Study on mechanism of multi-point site-specific mutagenesis.Science in China(series B)(press in chinese and English)

3)A novel, simple and efficient site-specific mutagenesis suitable for protein engineering.

Proc.Natl.Acad.Sci.USA or Nucl.Acid Res.

Work plan and time schedule (originally envisaged)

1991-1992: 1.To design the primers.

2.To synthesize these primers.

3.To construct the mutant library.

4.To identify the mutant library by dot-blot hybridization and DNA sequence analysis.

1992-1993: 1.To prepare of mutant Apr E genes

2.To construct a expression system.

3.To set up a convenient screening method

1992-1994: 1.To obtain several mutants with desired properties for instance, more thermostable, more stable at higher pH or resistant to oxidation.

2.To analyse the mutants by DNA sequencing.

Work plan and time schedule (actual)

Project landmarks, duration of individual tasks (use bar charts); evaluation criteria (publications, patents, services, training)

project landmarks	duration of individual tasks
Design of the primers	1991,9 ---1991,11
Synthesis of the primers	1991,11---1991,12
Construction of the mutant library	1991,12---1992,3
Identification of mutant library	1992,3 ---1992,7
Preparation of mutant Apr E genes	1992,9 ---1992,12
Construction of the expression system	1992,12---1993,4
Construction of the screening method	1993,4 ---1993,7
Screening of the mutants by expression	1994,9 ---1994,12
Analysis of the mutant by sequencing	1994,12---1995,5

Evaluation criteria:Interim report 1,2, terminal evaluation report and publications(No less than three articles are to be published).

NETWORKING

NONE

PUBLICATIONS

Now no less than three papers shall be published.

1.Construction of site-mutant subtilisin E pool with a set of oligonucleotides

SCIENCE IN CHINA (series B) (press in Chinese and English)

2.Study on mechanism of multi-point site-specific mutagenesis

SCIENCE IN CHINA (series B) (press in Chinese and English)

3.A novel,simple and efficient site-specific mutagenesis suitable for protein engineering.

Proc. Natl. Acad. Sci. USA. or Nucl. Acid. Res.