Primer Design and *in silico* Analysis of Endoglucanase Gene for *Bacillus* genus

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Abstract

Endoglucanase is an enzyme initiating hydrolysis of cellulose. The enzyme has been applied in various fields, such as medicine and food. Previous research has been isolated cellulolytic bacteria that are capable to produce endoglucanase, i.e. *Bacillus brevis* and *Bacillus cereus* from bekatul. Determination of bacterial isolate capability to produce endoglucanase can be conducted by gene detection. The detection method involves PCR using a universal designed primer to amplify the targeted gene. The aim of study was to design primers which amplify endoglucanase gene. The steps involved collecting data through NCBI, multiple sequence alignment using MUSCLE, design primer, *in silico* PCR and primer characteristics checking. Primer was designed by online program and literature. Template for primer design was *Bacillus* sp. (LN680001) consisting of 1500 bp. Primer design used three kinds of online program, i.e. Primer3plus, Primerquest tool, and primer BLAST. Every program resulted different length of product. Primer3plus and Primerquest tool produced 1124-1209 bp and 225-534 bp of product length, while primer BLAST produced 1403-1451 bp of product length. Primer based online program could not isolated endoglucanase gene completely. Based on literature study, primer CelF and CelR has been isolated endoglucanase gene, 1500bp. Analysis result showed literature studied primer produce length of product longer than online program primer. Primer endoglucanase gene was obtained 5' ATGAAACGGTCAATCTC for primer forward and 5' CTAATTTGGTTCTGTTC for primer reverse. Prediction of primer characterization based on annealing temperature, 41.2-42.1°C, and percentage of GC 46.3-48.1%. *In silico* PCR amplification analysis showed that primer proved specific to amplify the endoglucanase gene from *Bacillus* genus to produce complete gen, 1500 bp.

Keywords: design primer, *in silico*, endoglucanase gene, *Bacillus*

1. INTRODUCTION

Endoglucanase (EC 3.2.1.4) is a part of cellulose enzyme complex initiating cellulose hydrolysis process. The enzymes has function to degrade β -1,4-glikosidic bond specifically and produce short chain of cellulose (1,2). Endoglucanase was applied widely in various industries, such as brewing industry (3), biofuel (4), detergent (5) and animal feed (6). Other application endoglucanase are pharmaceutical industry, such as enzyme was used as therapy agent(7, 8, 9, 10).

Endoglucanase was produced for varied microorganism, including fungi and bacteria. Endoglucanase from bacteria is easier to isolate than from fungi (11). *Bacillus* is one of bacteria genus that capable to produce this enzyme, e.g. *B. cereus*, *B. licheniformis*, *B. subtilis*, *B. polymixa*, *B. amylolyquefaciens*, *B. pumilus*, *B. circulans* and *B. flexus*(12, 13, 14). Previous research has been

isolated bacteria producing endoglucanase from bekatul. Based on phenotypic identification, the bacteria were *B. Brevis* & *B. cereus*.

Exploration bacteria producing endoglucanase has been conducted. To study bacteria producing endoglucanase detailed is better start with the gene. Endoglucanase gene consists of 1500 bp approximately. Ozaki *et al.* (1991) and Han, *et al.* (1995), endoglucanase coding gene is 1389 and 1461, respectively. Isolation endoglucanase gene generally done by PCR method with certain primer. In this study, will be search primer to isolate endoglucanase gene by *in silico* approach and based on literature study.

2. MATERIALS AND METHODS

Instruments and Materials

Instruments used in this study were bioinformatics software online and offline for design primer, e.g. BioEdit, Primer3plus (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/>), Primerquest tool (<http://sg.idtdna.com/Primerquest/Home/Index>), Primer BLAST (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>), online OligoAnalyzer Tool (<https://sg.idtdna.com/calc/analyzer>), *in silico* PCR amplification (<http://insilico.ehu.eus/PCR/>), and Toshiba Laptop Satellite L745. The material used in this research was DNA sequence data of endoglucanase partial and complete gene from *Bacillus* genus downloaded in FASTA format from NCBI database.

Collecting Data

Endoglucanase genes were downloaded from GenBank web, NCBI (<http://www.ncbi.nlm.nih.gov/genbank/>). Endoglucanase genes partially and completely were from various *Bacillus* genus. Compiled endoglucanase gene sequences data was analyzed by MUSCLE in Bioedit program. From the analysis will be chosen one complete sequence as template to design primer (15, 16).

Primer Design

Primer was design using online software programs, e.g. Primer3plus, Primerquest tool, and Primer BLAST. Based three online programs resulted some pair primers with different length (15, 16).

Data Analysis

Primer designed was checked by *in silico* PCR amplification. Characteristic primer was analysis by online OligoAnalyzer Tool. The application examined the value of T_m (melting temperature) and GC ratio.

3. RESULTS AND DISCUSSION

Endoglucanase gene sequences data downloaded from NCBI database were presented in **Table 1**. There are fourteen gene sequences from various strain *Bacillus*, e.g. *B. subtilis*, *B. amyloliquefaciens*, and *B. megaterium*. The sequences are full gene and have high homology. *Bacillus* sp. was used as template, LN680001, to design primer endoglucanase gene.

TABLE 1.Endoglucanase gene sequence data mining

Strain <i>Bacillus</i>	Accession number	Sequence Length
<i>B. subtilis</i> IARI-SP-1	KF240847	1500
<i>B. amyloliquefaciens</i> IARI-SP-2	KF240848	1500
<i>B. megaterium</i> IARI-SP-9	KF240851	1500
<i>B. amyloliquefaciens</i> PSM 3.1	GU390463	1500
<i>B. subtilis</i> shu-3	HM470252	1500
<i>B. subtilis</i>	AY044252	2589
<i>B. subtilis</i> 168 trpC2	Z29076	1800
<i>B. subtilis</i>	X04689	2435
<i>B. subtilis</i> JA18	DQ116829	1500
<i>B. subtilis</i> BS-2	EU022560	1500
<i>B. subtilis</i> subsp. subtilis 168	NC_000964	1500
<i>B. subtilis</i>	D01057	1928
<i>B. subtilis</i>	X67044	2314
<i>Bacillus</i> sp.	LN680001	1500

Bacillus sp. as template was analyzed by three online design primer programs, e.g. Primer3plus, primerquest tool, and primer BLAST. Based on Primer3plus and primerquest tool were resulted five pair primers every program with various lengths. Primer3plus and primerquest tool resulted product length, 1124-1209 bp and 225-534 bp, respectively. Primer BLAST program produced ten pair primer with product length 1403-1450 bp. The longest product of each program was shown in **Table 2**.

TABLE 2. Pair Primer from online Primer design program

Program	Primer (5' → 3')	Product length
Primer3plus	Fx GGTACAGCTGAAGGGGATCA	1209
	Rx TCATTGTGAAGACGGAGCTG	
Primerquest tool	Fy ATGGAGCCAGGATGTGAATG	534
	Ry ACCGTGGTATTGCCGTTATT	
Primer BLAST	Fz CGTTATTGACAATGGGCGGC	1450
	Rz GGTTCTGTTCCCAATCAGT	

Based on Table 2, the longest product from primer BLAST was compared to primer from literature studied. Primer from literature studied has proved to isolate endoglucanase gene successfully. Product amplicon from both primer were 1311 bp for EN1F-EN1R primer and 1500 bp for CelF-CelR primer. It can be concluded that primer CelF and CelR can isolate endoglucanase gene completely. Primer annealing on the template was predicted in Fig. 1.

TABLE 3. Primer of endoglucanase gene from literature

No	Primer	References
1	EN1F 5' CCAGTAGCCAAGAATGGCCAGC EN1R 5' GGAATAATCGCCGCTTTGTGC	(17)
2	CelF 5' ATGAAACGGTCAATCTC CelR5' CTAATTTGGTTCTGTTCCC	(1)

>gi|724425628:1958638-1960137 Bacillus sp. genome assembly BS34ACh, chromosome : I

ATGAAACGGTCAATCTCTATTTTTATTACGTGTTTATTGATTACGTTATTGACAATGGGCGGCATGATAGCTTCGCCGGCATCAGCAG
CAGGGACAAAAACGCCAGTAGCCAAGAATGGCCAGCTTAGCATAAAAGGTACACAGCTCGTTAACCGAGACGGTAAAGCGGTACAGCT
GAAGGGGATCAGTTACACGGATTGCAATGGTATGGAGAATATGTCAATAAAGACAGCTTAAATGGCTGAGAGATGATTGGGGTATC
ACCGTTTTCCGTGACGCGATGTATACGGCAGATGGCGGTTATATTGACAACCCGTCCTGAAAAATAAAGTAAAGAAAGCGGTTGAAG
CGGCAAAAGAGCTTGGGATATATGTCATCATTGACTGGCATATCTTAAATGACGGTAATCCAAACCAAAATAAAGAGAAGGCAAAAGA
ATTCTTCAAGGAAATGTCAAGCCTTTACGGAAACACGCCAAACGTCATTTATGAAATTGCAAACGAACCAACCGGTGATGTGAACCTGG
AAGCGTGATATTAAACCATATGCGGAAGAAGTGATTTTCAGTTATCCGCAAAAATGATCCAGACAACATCATCATTGTGCGAACCAGTA
CATGGAGCCAGGATGTGAATGATGCTGCCGATGACCAGCTAAAAGATGCAAACGTTATGTACGCACTTCATTTTTATGCCGGCACACA
CGGCCAATTTTTACGGGATAAAGCAAACATATGCACTCAGCAAAGGAGCACCTATTTTGTGACAGAGTGGGGAAACAAAGCGACGCGTCT
GGCAATGGCGGTGATTCTTGTATCAATCGAGGGAATGGCTGAAATATCTCGACAGCAAGACCATTAGCTGGGTGAACCTGGAATCTTT
CTGATAAGCAGGAATCATCTCAGCTTTAAAGCCGGGGGCATCTAAAACAGGCGGCTGGCGGTTGTCAGATTTATCTGCTTCAGGAAC
ATTCGTTAGAGAAAACATTCTCGGCACCAAAAGATTCGACGAAGGACATTCCTGAAACGCCATCAAAAGATAAAACCCACACAGGAAAAT
GGTATTTCTGTACAGTACAGAGCAGGGGATGGGAGTATGAACAGCAACCAATCCGTCGCGAGCTTCAAATAAAAAATAACGGCAATA
CCACGGTTGATTTAAAGATGTCACTGCCCCGTTACTGGTATAAAGCGAAAAACAAAGGCCAAAACCTTTGACTGTGACTACGCGCAGAT
TGGATGCGGCAATGTGACACACAAGTTTGTGACGTTGCATAAACCAAGCAAGGTGCAGATACCTATCTGGAACCTGGATTAAAAAC
GGAACGTTGGCACCAGGAGCAAGCACAGGGAATATTCAGCTCCGTCTTCACAATGATGACTGGAGCAATTATGCACAAAGCGGCGATT
ATTCTTTTTTCAAATCAAATACGTTTAAACAACGAAAAAATCACATTATATGATCAAGGAAAACCTGATTTCTGGGAACAGAACCAAA
TTAG

FIGURE 1. Prediction of annealing primer of Fz-Rz and CelF-CelR based on LN680001 template. Fz-Rz primer was indicated by underline letter, while CelF-CelR was indicated by box letter

Both primers were checked by *in silico* PCR amplification. Fz-Rz primer gave no result, while CelF-CelR primer gave a 1500 product. The product shown a band only for *B. amyloliquifaciens* and *B. subtilis*. Characteristic primer based on Oligoanalyzer tools shown annealing temperature and GC ratio. Characterization annealing temperature and GC ratio each primer respectively was 46.3°C and 41.2% for CelF, and 48.2°C and 42.1% for CelR.

4. CONCLUSION

Endoglucanase gene from *Bacillus* genus can be isolated using primer CelF, 5'ATGAAACGGTCAATCTC for forward, and CelR, 5' CTAATTTGGTTCTGTTCCC for reverse.

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