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Short Communication

Molecular cloning and sequencing of penicillin G acylase from *Shigella boydii*

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In this study, 290 non-*Escherichia coli* Enterobacteriasea that were isolated from environmental and clinical specimen, were sent to the laboratory for examination with routine microbiological tests for identification of isolates. After identification, non-*E. coli* isolates were inspected by PCR for existence of penicillin G acylase (PGA) gene. Then, a PGA positive strain (*Shigella boydii*) from clinical specimens was selected for further analysis. First, DNA was isolated and PCR reactions were conducted using primers based on conserved region of PGA genes. The PCR reaction resulted in amplification of a specific product with expected length. The PCR product was cloned in pGEM-T Easy vector. Sequencing revealed that the gene, composed of encodes a polypeptide of 846 amino acid residues. Analysis of obtained sequence against databases showed the highest homology (about 96%) with the PGA gene reported from *S. boydii*.

Key words: Penicillin G acylase, *Shigella boydii*, identification.

INTRODUCTION

Penicillin G acylase (PGA) is an enzyme that catalyzes the deacylation of β-lactam antibiotics (Savidge et al., 1975). It has been used to hydrolyze benzyl penicillin to generate phenyl acetic acid and 6-aminopenicillanic acid. 6-Aminopenicillanic acid is substrate for production of many semi-synthetic penicillins (Valle et al., 1991). Also PGA is present in sevearal bacteria (Yang et al., 2006).

In view of current environmentally situations, microbial enzyme transformation processes are generally preferred over the conventional chemical conversion process. The advantages include less chemical load on the environment and higher efficiency (Vandamme and Voets, 1974). β-Lactam antibiotics, like penicillins and cephalosporins represent one of the major world's biotechnology markets (annual sales of $15 billion, and about 65% of the total antibiotics market) (Elander, 2003).
The aim of this study is to isolate a non-*E. coli* Enterobacteriasea PGA by PCR and cloning of the PGA into *E. coli*.

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MATERIAL AND METHODS

Chemicals, enzymes, and strains

Restriction endonucleases and *Taq* DNA polymerase were obtained from Fermentas (Lithuania). *E. coli* DH5α (Invitrogen, Netherlands) was used as host strains for the genetic cloning and expression. The plasmid pGEM-T easy vector (Promega, USA) was used for cloning.

Isolation of the *Shigella boydii*

The *S. boydii* strain as a member of non-*E. coli* enterobacteriasea used in this study was provided by our laboratory collection. This bacterium was collected from environmental and clinical specimens during routine screening. 290 Enterobacteriaceae were collected from environmental and clinical specimens. Isolates were cultured in LB media and were used for DNA Extraction. In this study, we assumed all Gram negative, oxidase negative bacilli as Enterobacteriaceae and did several tests for *S. boydii* diagnosis including indole, methyl red, Voges-Proskauer, and citrate (IMVIC tests).

DNA extraction

Bacteria was cultured on LB broth 24 h at 37°C and centrifuged at 9000 rpm for 3 min. The cell pellets was dissolved in lysis buffer (10

Table 1. The sequences of primers were used for amplification and nested PCR confirmation of PGA gene (ORF = open reading frame).

Primers	Code	Sequence
Full ORF primers	PGA F1	5'- ATGAAAAATAGAAATCGTATGATC-3'
	PGAR1	5'- TCTCTGAACGTGCAACACTTC-3'
Nested primers	PGA F2	5'- TGTCGCGATGATATTTGTG-3'
	PGAR2	5'- GCGGGATTTAGCGTAAGC-3'

mM Tris, 10 mM EDTA and 1% SDS) and incubated with protein kinase (10 mg/ml) 24 h at 40°C. Then, the samples were incubated with Rnase A for 30 min followed by phenol chloroform extraction. DNA was precipitated with 2 volume of ethanol 100% washed by alcohol 70%, dried and dissolved in TE buffer.

PCR

The PCR reaction mixtures included Taq DNA polymerase buffer, with each deoxynucleoside triphosphate at a concentration of 200 µmol/L, each primer at a concentration of 1 µmol/L, and 1.5 unit of *Taq* DNA polymerase in a total volume of 25 µL. DNA amplification was achieved with an Eppendorf thermocycler utilizing the following method: first denaturation for 4 min at 94°C, followed by 30 cycles of denaturation 30 s at 94°C, annealing 60 s at 68°C, and extension 150 s at 72°C, with a final extension at 72°C for 5 min. DNA templates were added to a final concentration of 1 ng µL–1. The PCR products were verified by electrophoresis on 1% agarose gel stained with ethidium bromide. Primers PGA-F and PGA-R (Table 1) were used to amplify the PGA gene. The sequence of primers corresponds to the sequence of PGA gene reported from *E. coli*. The primers were produced by Prime Co (Italy).

Cloning and sequencing of *S. boydii* PGA gene

The amplified PCR product were purified by passage from silica column and ligated into pGEM-T easy vector (Promega USA). The ligation product was transformed into *E. coli* DH5α and cultured on LB agar media including ampicillin (100 mg/ml), IPTG (1 mM), and X-GAL (1 mM). Then plasmid extraction was achieved from white colonies and cloning was verified by digestion with restriction enzymes.

RESULTS

Amplification of PGA gene and sequencing

The genomic DNA from different non-*E. coli* enteric bacteria was extracted and utilized as the PCR template. Primers PGA-F and PGA-R were designed on the basis of the PGA sequences reported from *E. coli*. A total of 290 genomic DNA samples from different non-*E. coli* enteric bacteria were used as the target templates to amplify the PGA gene. As shown in Figure 1, PCR amplification resulted in a single specific DNA fragments (about 2350 bp) that suggested PGA genes were successfully amplified. The results were verified by nested-PCR and RFLP methods.

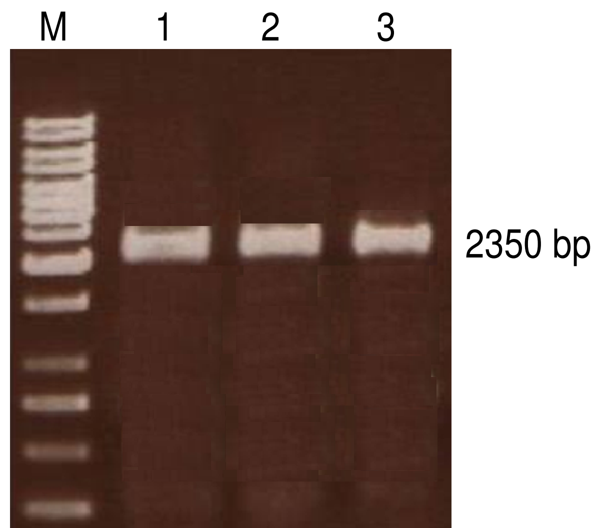

Figure 1. PCR amplification of PGA gene from *Shigella boydii* isolates. Lane M, molecular size markers; lanes 1, 2 and 3, amplified products with just a single band each.

Cloning and sequencing of penicillin G acylase genes

After initial confirmation, the PCR product were ligated into the pGEM-T easy TA cloning vector and the recombinant plasmids were examined via the blue-white colony and PCR methods. Penicillin G acylase genes in the recombinant plasmid were confirmed by sequencing. The structural gene is 2538 bp which encoded a polypeptide of 846 amino acid residues. Sequence analyses revealed that the sequence obtained from the *S. boydii* isolate has high homology (about 96%) with other previously reported PGA genes.

DISCUSSION

For bulk production of β-lactam antibiotics, significant progress has achieved in the past two decades. Industries that produce β-lactam antibiotics have presented PGA biocatalysts that replaces multistep conversion processes with cheap and well enzymatic conversion, which has an efficiency of about 80 – 90%. The produc-

tion of β-lactam antibiotics by PGA-mediated method is a new direction for antibiotics industries with safer and cleaner environment (Elander, 2003). Apart from β-lactam hydrolysis, other developments have resulted in multiple applications of PGA, including peptide synthesis, resolution of racemic mixture, enantioselective acylation, etc (Olsson et al., 1985).

In this work, a total of, 290 non-*E. coli* Enterobacteriaceae bacteria were investigated. All bacteria were diagnosed by routine microbiology methods. DNA extraction was done successful for all bacteria, but there was only one non-*E. coli* that had PGA, *S. Boydii*, and was used for cloning. DNA sequencing result showed that the gene encoding penicillin G acylase in *S. boydii* have an open reading frame of 2538 bp encoding 846 amino acids. Analysis of sequencing result revealed that this gene contain 96% homology with that reported in NCBI (Gene Bank Accession). The result of our study indicated high level conservation of PGA gene at least among Enterobacteriasea. The high conservation of PGA gene in different bacteria (Garcia and Busea, 1986) may implicate the importance of this gene for these bacteria. Also *S. boydii* can be utilized for PGA isolation which can benefit the pharmaceutical industries.

Results revealed that the importance of the polymerase chain reaction method, with primers specific to the target gene. Also it is a very sensitive and specific method that can be used for screening of PGA genes from different samples and feasibility of utilizing various bacteria as PGA resources.

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