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Isolation, Identification and Cloning of the Penicillin G Acylase Gene from *Shigella boydii* Cloned in *Escherichia coli*

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Abstract: Penicillin G Acylase (PGA) is an important enzyme in the bulk pharmaceutical industry. It is one of the most important industrial enzymes for the production of semi-synthetic penicillin. PGAs have been found in numerous bacteria and fungi. The PGA gene has been isolated from different organism displays distinct biochemical properties which may be important for industrial aspects. In this study, 250 non *E. coli*, Enterobacteriasea that were collected from environmental and clinical samples, Transported to the laboratory and subjected for routine microbiological tests for identification of isolates. After identification, non *E. coli* isolates were investigated by PCR for processing of PGA gene. In this method, a PGA positive strain from an isolate that identified as *Shigella boydii* by standard microbiological test. DNA extracted from *Shigella boydii* entered in PCR reactions using primers designed on conserved region of PGA genes. The gene were cloned in pGEM-Teasy vector and submitted for sequencing. The gene encoding a Penicillin G acylase from *Shigella boydii* isolate contain an open reading frame of 2534 nucleotide encoding 796 amino acids. Analysis of sequencing result showed that this gene contain 98% homology to previously reported PGA from a *Shigella boydii* strains.

Key words: PGA, PCR, *Shigella boydii*, Enterobacteriasea, cloning

INTRODUCTION

Penicillin G acylase (PGA) is an important enzyme in the bulk pharmaceutical industry (Vireden, 1990). 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).

The PGA gene of *Escherichia coli* encodes a penicillin G acylase (PGA). Penicillin G acylase (PGA, EC 3.5.1.11) is a type II penicillin acylase that hydrolyzes Penicillin G to 6-aminopenicillanic acid (6-APA) and phenyl acetic acid (PAA) (Buesa *et al*., 1999). It is one of the most important industrial enzymes for the production of semi-synthetic penicillin (Verhaert *et al*., 1997). PGAs have been found in numerous bacteria and fungi and the PGA of *Escherichia coli* is characterized (Ohashi *et al*., 1988).

The PGA enzyme is synthesized as an inactive preprotein that contains a leader peptide directing the protein is destination and a spacer peptide separating the α and β subunits (Mahmood *et al*., 1991). Formation of active PGA includes a series of post translational steps via translocation and periplasmic processing and folding that are unusual for prokaryotic proteins (Sudhakaran *et al*., 2007). Acylase type I is specific for PenV and have four subunit and 35 KD molecular weigh. Acylase type II have α subunit with 16-22 KD molecular weigh and β subunit with 54-66 KD molecular weigh (Diaz *et al*., 2001). Acylase type III (Ampicillin acylase) has 2 subunit with 72 KD molecular weigh.

The routine method for PGA production is from the *Escherichia coli* ATCC1105. But this method produce very low rate PGA and have a limitations, like existence of PAA to the culture permanently that it is very expensive. It seems that production by recombinant protein technology can have PGA enzyme more and cheap.

Betalactame antibiotics, penicillin and cephalosporin produce by fermentation reactions and penicillium or cephalosporium fungi.

Pen G, Pen V and Cef C are the primary substrates for generation of penicillin and cephalosporin antibiotics groups. Also, Acyle group in the 6-Amino position of penicillin and Acyle group in the 7-Amino of cephalosporin antibiotics include side chain of molecule. The side chains of Pen G, Pen V and Cef C are Phenyl

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acetyl, Phenoxy acetyl and amino adipyl. This side chains were deleted by amid bond fracture and form 6APA (6-Amino penicillanic acid) for Pen G, V and 7-ACA (7-Amino cephalosporanic acid) for cephalosporin.

The semi-synthetic Penicillins or cephalosporins have resistance to Betalactames and so have the benefits more than primary molecules (Diaz *et al*., 2007). The generation of Betalactame antibiotics need for deacylation of produced Penicillins or cephalosporins by fermentation methods and the enzymatic methods better than chemical kind (Horton, 1993). Because chemical reagents are expensive and have environmental contamination probability (Horton, 1993). Enzymes that can catalyze Betalactame molecules are called Acylase or Amidase (Schumacher *et al*., 1986). The main method for generation of PGA is Random mutagenesis, Screening selection and directed mutagenesis (Robas and Branlant, 1994). Bacteria was found by screening selection and in the other process the gene is cloned by recombinant DNA and is produced in mass concentration (Sriubolmas *et al*., 1997).

In this study, *E. coli* and other species of Enterobacteriasea were investigated for PGA.

Members of genera belonging to the Enterobacteriaceae family have earned a reputation placing them among the most pathogenic and most often encountered organisms in clinical microbiology. These large Gram-negative rods are usually associated with intestinal infections, but can be found in almost all natural habitats. They are the causative agents of such diseases as meningitis, bacillary dysentery, typhoid and food poisoning. As well as being oxidase negative, all members of this family are glucose fermenters and nitrate reducers. In most cases, the pathogenicity of a particular enteric bacterium can be determined by its ability to metabolize lactose. Non-utilizers are usually pathogenic while the lactose utilizers are not. Because many different species in this family, can cause similar symptoms, biochemical tests are crucial to the identification, diagnosis and treatment of infection.

Purposes:

- Screening of members of Enterobacteriaceae from environmental and clinical specimens for PGA by PCR.
- PGA cloning from non *E. coli* bacterias and sequencing of the gene.

MATERIALS AND METHODS

Specimens and sampling procedure were Enterobacteriaceae from environmental and clinical sources. Then PGA positive bacteria were selected. PCR method was used for test processes (Fig. 1).

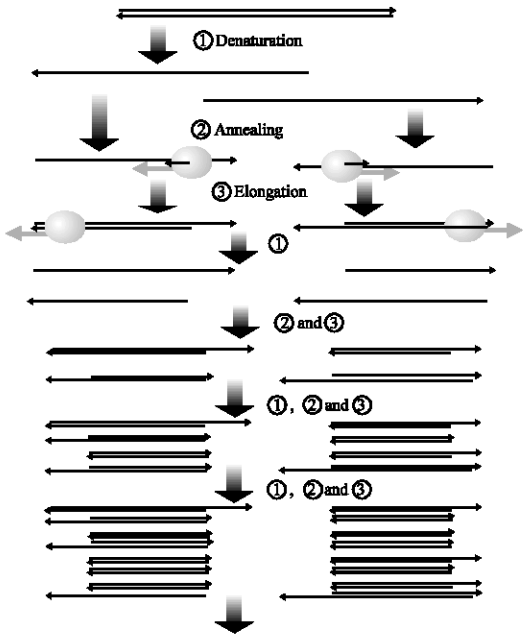

Fig. 1: Schematic drawing of the PCR cycle. (1) Denaturing at 94-96°C. (2) Annealing at ~65°C (3) Elongation at 72°C. Four cycles are shown here

Two hundred and ninty Enterobacteriaceae were collected from environmental (Water, soil) and clinical specimens. All of bacteria were distincted by the using transport media and EMB and Macongi agar and differential Medias. Isolates were cultured in LB media and were used for DNA Extraction.

DNA extraction: First of all, bacteria were dissolved in Lysis buffer with SDS and EDTA. Then all of them were incubated with protein kinase 24 h. Also samples were incubated with Rnase 30 min and were extracted with phenol chloroform. DNA was precipitated with Ethanol 100% and was washed and dried by Alcohol 70% and was dissolved in TE buffer.

Study of bacteria's DNA for PGA by PCR method: The gene sequencing of PGA was obtained from NCBI gene bank. Primers was provided by Italy Prime Co and was used in PCR reactions. In the mention of preparation of the best reaction, *E. coli* (ATCC 11105) was used. Electrophoresis of PCR product with size marker was achieved in Gel Agarose and was stained by Etidium bromide and photos were provided by Gel doc instrument (Fig. 2).

PGA gene cloning from *Shigella boydii*: First of all PCR products were collected after 4 times PCR. Then products were purified by passage from silica column and entered

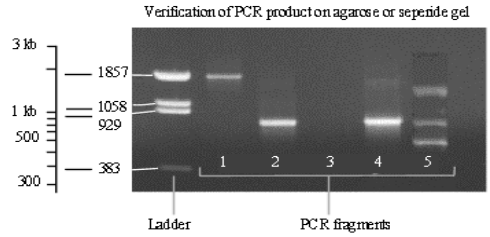

Fig. 2: Verification of the PCR product on gel

to Ligation reaction with pGEM vector (provided by Promega Co). Product of Ligation transformed in *E. coli* type DH5a and was cultured in the LB media containing Ampicillin, IPTG and X-GAL. Then plasmid extraction was done from colonies and was acquainted by digestion with restricted enzymes and PCR method.

RESULTS AND DISCUSSION

Many factors are responsible in the enzymatic conversion of primary Betalactames to intermediate substances:

Reaction factors: PH, temperature, ion concentration, dissolver:

- Enzyme stability.
- Reactant factors constancy.
- Enzyme catalytic activity.

The need for innovative application-specific thermo stable DNA polymerases is growing rapidly. Q biogene has developed a comprehensive selection of enzymes covering all major PCR applications. Several of these polymerases are completely unique and offer important advantages and it is better that use for further investigations.

In the mention of objectives, first of all, 290 Enterobacteriaceae (160 *E. coli*) collected from 350 specimen. Among non *E. coli* bacteria, *Shigella boydii* had PGA. All bacteria were distinguished by standard tests, Glucose or Lactose fermentation, Motility, citrate utility, etc.

DNA extraction was successful for all bacteria, but there was only one bacterium that had PGA, *S. boydii* and was selected for cloning.

In the second process, after PCR and enzymatic section, PGA cloning from *Shigella boydii* and sequencing of the gene was done.

The gene encoding a Penicillin G acylase from *Shigella boydii* contain an open reading frame of 2630 nucleotide encoding 886 amino acids. Analysis of sequencing result demonstrated that this gene contain 98% homology to previously reported PGA from a *Shigella boydii* strains. And there was no significant difference with previous studies ($p>0.05$) (Calleri and Massolini, 2004).

The high conservation of PGA gene in different bacteria may implicate importance of this gene for these bacteria. Also *Shigella Boydii* can be used for PGA isolating and utilization in pharmaceutical industries.

CONCLUSION

This review has focused on the development and applications of PCR reactions for PGA gene cloning From *Shigella boydii*, it is clear that the PGA based-stationary phases are capable of producing substances with potential high purity (and consequently high added value). The molecular modeling studies gave more insights into PGA-substrates interactions and this information could expand the application of PCR as a cheap and high speed technique for pharmaceutical and biological processes.

Therefore, PCR can be used as a tool for Expression and Regulation of the Penicillin G Acylase Gene from *Shigella boydii* Cloned in *Escherichia coli*. And this method increases the rate of biological substances. There is lot of improvement possible; however the promising results obtained with PCR have opened a new road for biocatalysts (Calleri and Massolini, 2004).

The gene encoding a Penicillin G acylase from *Shigella Boydii* isolate have an open reading frame of 2540 nucleotide encoding 846 amino acids. Analysis of results showed that this frame contain 98% homology with previously papers about PGA from a *Shigella boydii* strains.

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