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Artikel

Cloning and Characterization of Gene Encoding Thermostable Enzyme from Domas Crater

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Abstract: This research has been carried out an analysis of the gene encoding thermostable enzymes from natural samples of Domas Crater by using a metagenomic approach. The purpose of this study was to identify the gene encoded thermostable enzyme from natural samples of the Domas Crater. In this study, genes were amplified through random PCR technique from a collection of community DNA that had been isolated previously using a metagenomic approach. Random PCR of total community DNA produced DNA fragments with a size of about 1 kb, and then ligated to the pJET1.2/blunt vector and inserted into *E. coli* TOP10 for cloning process. The recombinant plasmid was then analysed using restriction enzymes *BamH*I and *Eco*RI to confirm the presence of the gene inserted in the cloning vector. Homology analysis of the DNA sequences of the cloned genes showed five groups of proteins that were similar to the ABC Transporter permease protein from archaea with an identity of about 76 – 96%. The grouping of these proteins is shown by the constructed phylogenetic tree.

Keyword: *Gen, Cloning, Enzyme, Domas Crater*

1. Introduction

Currently, enzymes are widely used as biocatalysts in the industry [1]. According to Markets and Markets, the demand for enzymes will grow in 2026 from 2020. This is due to the increasing human awareness of the impact of using chemicals on the environment [2]. So, a lot of research is currently being done to apply green chemistry, namely efforts to design environmentally friendly chemicals and do not cause pollution problems [3]

Based on the concept of green chemistry, the use of enzymes is an effort that is in line with this concept. This is because enzymes are chemical compounds that easily decompose in the environment, so they do not cause new problems for the environment [4]. Enzymes are chemical compounds that are selective in producing compounds. One of them is thermostable enzymes [5]. Lipase is an example of a thermostable enzyme commonly used in the manufacture of detergents [6]. Its ability to catalyze the hydrolysis reaction of triglycerides, so that lipase acts as a surfactant [7]. In addition to being selective in producing a compound, thermostable enzymes have the ability to survive at high temperatures, namely at a temperature of 50 °C [8].

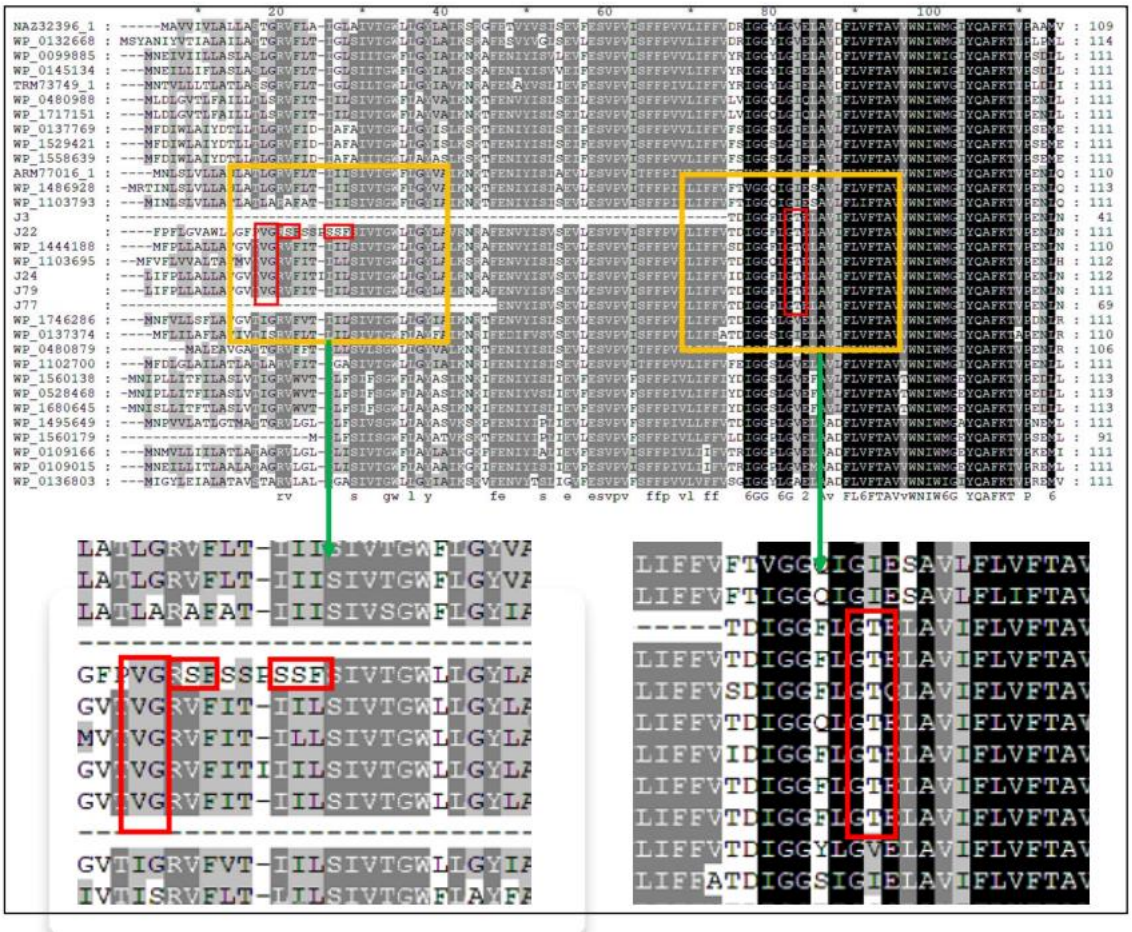
From their ability to survive at high temperatures, thermostable enzymes are chemical compounds derived from microorganisms that live in areas that have high temperatures [9], such as craters, hot springs, volcanoes, etc. [10]. In this study, the thermostable enzyme encoding gene was isolated from natural samples of domas crater, with a temperature of around 92-95 °C [11]. Based on the temperature, it is possible for the existence of microorganisms that are stable at high temperatures. Where the microorganism has a gene encoding a thermostable enzyme

2. Material and Method

2.1. DNA amplification

DNA isolated from Domas Crater [16] was amplified using PCR. The primers used are PTO 611 F: 5'- CCG GCC GCT AGC CTT GAT AAT AAA AAG GAG TAT CT -3' and PTO 611 R: 5'- CGC GCG TCG ACT TAT TCA AAA ATT TTT ATG TAT G -3'. The solutions used were ddH_2O, PCR buffer, dNTPs, Primers F and R, $MgCl_2$, *Taq* Pol, and DNA templates. The PCR reaction was performed at 94 °C for 4' for first denaturation, 94 °C for 30 s for denaturation, temperature gradient 45 °Cto 55 °C for 30s for annealing, extension 72 °C for 1,5'on 30 cycles, final extension 72 °C for10', and cooling at 12 °C for 10'. The PCR results were then electrophoresed to determine the size of the amplification combination obtained. Electrophoresis was carried out by inserting 5µL of PCR results which had been added with 2µL of loading dye into the agarose well. Then connected to a 100 Volt electric current for 25 minutes. The results of the electrophoresis were then viewed using a UV transilluminator [17].

Figure 1. Amino acid homology



2.2. Cloning

DNA ligated with pJET/1.2 blunt plasmid using T4 ligase forms plasmid DNA. Samples were transformed into TOP10 *E. coli* cells using the 42°C heat shock method. Transformants were incubated at 36°C overnight using Luria Bertany media (1% tryptone, 1% NaCl, 0.5% yeast extract, and 2% agar powder). Plasmid DNA was isolated using 3 solutions. The first solution is Tris-HCl, EDTA, and sucrose. Solution 2 consisted of 1% SDS and 0.2N NaOH, and solution 3 contained potassium acetat and glacial acetate.

The isolated plasmid DNA was analyzed using restriction enzymes, *Bam*H1 and *Eco*R1, respectively. Where the respective composition ratios are 12:1:2:5 for NFW: restriction enzyme: enzyme buffer: plasmid DNA.

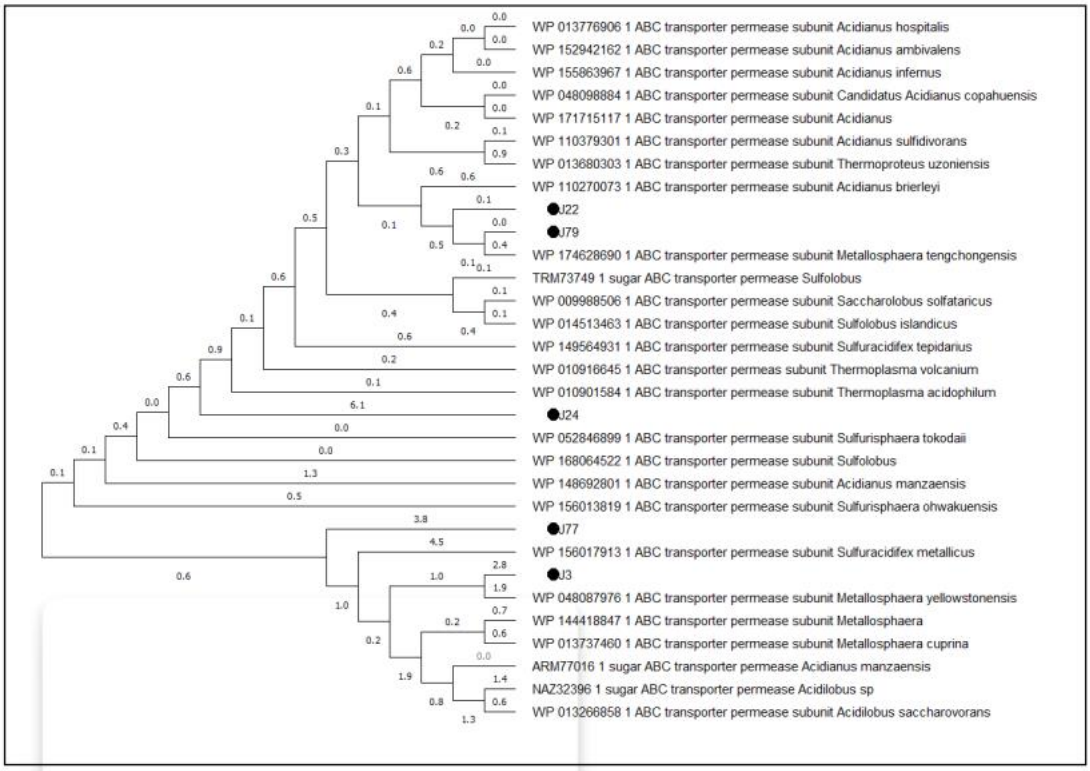
Samples were incubated for 24 hours at 37°C. The incubation results were then electrophoresed and viewed using UV light.

2.3. Homology analysis

Homology analysis of the sample was carried out in several stages as follows: The nucleotide sequences of the sample were analyzed using Bioedit, Editseq, and notepad software to fixed the DNA sequences. Furthermore, DNA sequence homology analysis was carried out using the BLAST X (Basic Logical Alignment Search Tool X) method on the NCBI page. The results from BLAST X will get the percentage of similarity of the sample to the enzymes that already exist on the National Center for Biotechnology Information (NCBI) page. In addition, the sample amino acid sequence was also obtained in the BLASTX results [18].

Amino acid sequence homology analysis was carried out using Clustalx and GENEDOC software. Amino acid homology analysis was performed by comparing the amino acid sequence of the sample with the 10 amino acid sequences of the enzyme that had the highest similarity to the sample [19]. Phylogenetic tree was performed using MegaX software.

Figure 2. Amino acid homology



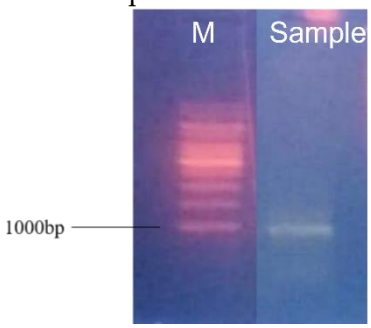
3. Result and Discussion

3.1 PCR product and recombinant plasmid

Total community DNA was successfully amplified by PCR technique using a pair of primers PTO611F and PTO611R to produce DNA fragments measuring about 1000 bp. The DNA fragment resulting from this PCR was visualized using agarose gel in Figure 1. The DNA fragment from this PCR then ligated into the pJET1.2/blunt cloning vector and inserted into E. coli TOP10 cells for the cloning process.

Rapid analysis using the size screening technique of *E. coli* transformant colonies showed the presence of several DNA bands with a size of more than 3 kb (figure 2). This suggests that *E. coli* transformants carry vectors that have inserted DNA fragments. The next rapid analysis is to use colony PCR. In this technique, transformant colonies that have been confirmed positive carrying recombinant plasmids are selected for direct PCR. In this PCR process, PTO611F and PTO611R primers were used. The PCR product of this colony is shown in Figure 3. From Figure 3, there are DNA fragments with a size of about 1000 bp. This suggests that the transformant colony carried a DNA fragment of about 1 kb in size.

Fig. 3. Electropherogram of DNA amplicon with PTO 611 F and PTO 611 R primers



To ensure the presence of recombinant plasmids in *E. coli* cells, the plasmid was isolated. Plasmids that have been isolated from E. coli host cells were further confirmed using restriction enzymes *Eco*RI and *Bam*HI. According to the pJET1.2/blut vector map, it shows that the vector lacks the cutting side of the restriction enzymes *Eco*RI and *Bam*HI. The results of the recombinant plasmid cutting showed that the plasmid was cut into one linear strand measuring about 4 kb (Fig. 4). This indicates that the DNA fragment that has been inserted into the pJET1.2/blunt vector has one cutting side for the restriction enzymes *Eco*RI and *Bam*HI. Based on the size of the truncated plasmid, it was confirmed that a DNA fragment of about 1 kb in size was inserted into the vector.

Fig. 4. Electropherogram size screening of DNA plasmid

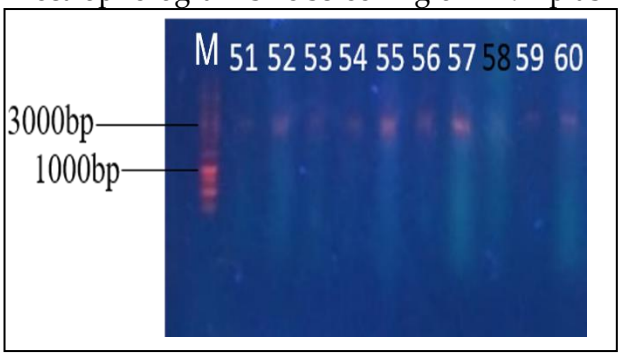
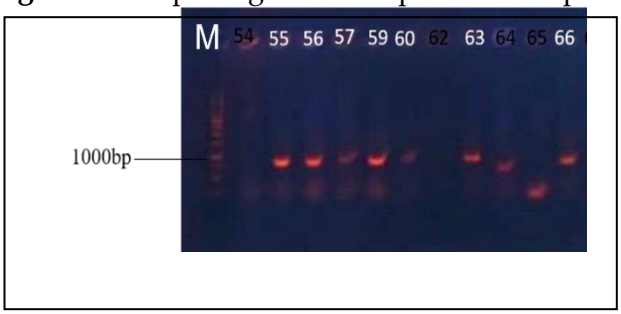


Fig. 5. Elektropherogram of amplicon DNA plamid



3.2 Homology

Homology analysis the DNA sequence of 5 samples performed using the online program BLASTX from NCBI is shown in Table 1. Most of the samples had the closest homology to the ABC Transporter permease of the archaea group *Metallosphaera sedula* with identities between 80 -96%. There is one sample that has the closest homology to the ABC Transporter permease of archaea *Acidianus sulfidivorans* with an identity of 76.84%. The data suggest that these samples varied within one group and within different genera.

Homology analysis was also performed using the clustalx program to align the amino acid sequence of the sample with the corresponding protein amino acid sequences from the GenBank. The alignment of the amino acid sequences is visualized with the genedoc shown in Figure 5. From the genedoc data, there are different amino acid sequences from the sample amino acid sequences compared to other amino acid sequences. This difference is indicated by the red rectangle in the figure. Interestingly, there is one T amino acid that distinguishes all samples from other amino acid sequences found in the conserved region, the amino acids for the others at that position as general are I or V.

Fig. 6. a. Electropherogram DNA plasmid analysis using *BamH1* enzyme, b. Electropherogram DNA plasmid analysis using *ecoR1* enzyme

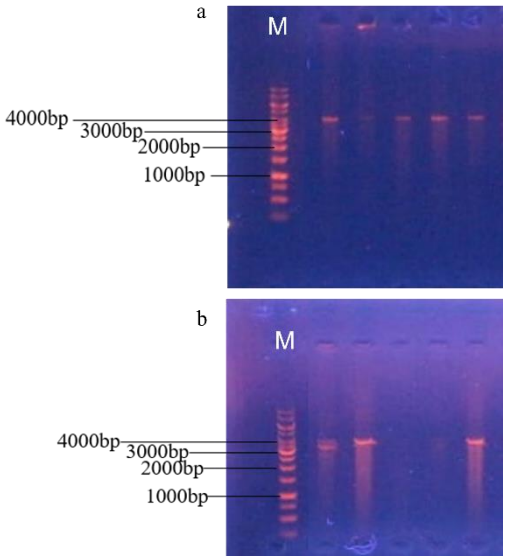


Table 1. BLAST alignment results on samples that have been sequenced

Sample	Description	Total scor	Ident.	Accession
J3	ABC transporter permease subunit [*Metallosphaera sedula*]	386	93.98%	WP_14441887.1
J22	ABC transporter permease subunit [*Metallosphaera sedula*]	379	87.29%	WP_14441887.1
J24	ABC transporter permease subunit [*Metallosphaera sedula*]	367	81.41%	WP_14441887.1
J77	ABC transporter permease subunit [*Acidianus sulfidivorans*]	285	76.84%	WP_110379301.1
J79	ABC transporter permease subunit [*Metallosphaera sedula*]	289	95.83%	WP_14441887.1

3.3 Phylogenetic tree

The phylogenetic analysis of the samples was carried out by constructing a sample phylogenetic tree with the closest suitable amino acid sequence shown in Figure 7. From this tree the samples were distributed in 5 different groups. Each creates a separate branch, creating its own branch. This tree also suggests significant differences in the amino acid sequence of Blastx results from NCBI. J79 from blastx results showed the highest identity with *Metallosphaera sedula* with 95.83% identity, while in the tree it showed closeness to *Metallasphaera tengchongensis*, as well as J22. J3 has the closest homology to *Metallosphaera sedula* with an identity of 93.98%, apparently having a closer relationship with *Metallosphaera yellowstonensis*. J24 and J77 each form separates unrelated branches that are far from the closest protein amino acid sequence. These phylogenetic tree data suggest that all sample proteins are diverse proteins. These data suggest that the five samples are different proteins, and are novel proteins that are different from the previous closest proteins.

3.4 ABC Transporter Permease

Adenosine Triphosphate (ATP) Binding Cassette (ABC) Transporter is an integral membrane protein that uses energy from the ATP hydrolysis process to move substrates across the membrane [12]. The ABC transporter is composed of 2 domains, namely the transmembrane domain (TMD) and the nucleotide-binding domain (NBD). TMD plays a role in recognizing the substrate and has a changeable conformation and transport of the substrate through the membrane. In addition, TMD also has another name for membrane spanning-domain or membrane integral. While NBD is an ATP binding site that has an animated amino acid sequence [13].

Based on their domain, ABC transporters have an important role in the uptake, export, and osmoregulation of substrates. Compared to other types of transport cases, ABC transporters can move a wide variety of substrates from inorganic and molecular organic substrates such as amino acids, sugars, nucleotides, vitamins, metal clusters, to larger organic compounds such as peptides, fats, and polysaccharides. ABC transporters also play a role in several important processes such as nutrient uptake, lipid trafficking, excretion of drugs and antibiotics, presentation of secretory macromolecules to cytotoxic T cells, and regulation of cell volume [14]. Currently, ABC transporters are used in the pharmaceutical industry, whereas ABC transporters are used in the development of multidrug-resistant. One of them is in the development of cancer drugs, ABC transporter plays a role in drug mobilization. So that in the future the ABC transporter is expected to be able to become a multidrug-resistant medium [15].

4. Conclussion

The metagenomic approach to obtain new protein-coding genes has been successfully carried out on samples of the Domas Crater. Five new protein fragments have been cloned

directly from natural samples of Domas Crater. These protein fragments were confirmed to be part of novel proteins.

Daftar Pustaka

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