Available online at website: www.bbp4b.litbang.kkp.go.id/squalen-bulletin

Squalen Bulletin of Marine & Fisheries Postharvest & Biotechnology, 8 (3), 2013, 129-137

Transposon Insertion Phenomenon during Cloning of a Partial Fragment Derived from Metagenomic DNA Isolated from Deep-Sea Water and Sediment of Kawio Island, North Sulawesi

Fenomena Insersi Transposon pada Proses Kloning Fragmen Parsial dari DNA Metagenom yang Diisolasi dari Air dan Sedimen Laut-Dalam Perairan Pulau Kawio, Sulawesi Utara

Mochamad Untung Kurnia Agung[1]*and Maelita Ramdani Moeis[2]

[1]Department of Marine Science, Faculty of Fisheries and Marine Sciences, University of Padjadjaran (UNPAD), Sumedang
[2]School of Life Science and Technology, Bandung Institute of Technology (ITB)
*Correspondence Author: mochamad.untung@gmail.com

Article history:
Received: 3 April 2013; Revised: 18 November 2013; Accepted: 19 November 2013

ABSTRACT

Transposon is well-known as mobile element found abundant both in prokaryote and eukaryote genomes. In bacteria, transposon (famous name of a transposable DNA) could jump from chromosome to plasmid and its contrary. One type of transposons in bacteria known as *insertion sequence* (IS), it does not contain any additional genes except a gene encoding transposase, an enzyme that correlated to transponsition activities. The finding of transposon insertion unfortunately found during cloning of a fragment derived from deep-sea metagenomic DNA in this research. In the initial, this research was aimed to clone and characterize the á-amylase encoded gene derived from metagenomic DNA isolated from deep-sea water and sediment of Kawio Island, North Sulawesi. Metagenomic DNA has been isolated from deep-sea water and sediment and by using Whole Genome Amplification (WGA) technique, the DNA it could be increased in quantities to 146,31 ng for each 1 ng of metagenomic DNA. A fragment of ~1000 bp in length was obtained by using touchdown PCR method. The presence of a transposon in this DNA fragment is proposed as a hypothesis for losing ~700 bp leaving just 310 bp cloned sequence. Analysis of sequencing result showed a highest similarity between this 310 bp partial fragment with a replication protein (Rep) encoded gene from *Pseudomonas putida* (Query Coverage: 88%; Max. Identity: 80%, Positive: 86%) and this protein is known to be involved in plasmid replication where transposase encoding genes known usually presence together with this gene (Rep gene) in a bacterial plasmid.

Keywords: transposon, metagenomic, partial fragment, cloning, deep-sea

ABSTRAK

Transposon yang dikenal sebagai *mobile element* ditemukan melimpah pada genom prokariot maupun eukariot. Pada bakteri, transposon dapat berpindah dari kromosom ke dalam plasmid dan atau sebaliknya. Salah satu jenis transposon yang ditemukan pada bakteri yang dikenal sebagai *insertion sequence* (IS), diketahui tidak mengkode gen tambahan lain kecuali gen pengkode transposase, yaitu enzim yang berperan pada aktivitas transposisi dari suatu transposon. Penemuan fenomena aktivitas insersi (transposisi) dari suatu transposon secara tidak sengaja ditemukan pada saat proses kloning suatu fragmen parsial yang berasal dari DNA metagenom laut-dalam pada penelitian ini. Pada mulanya, penelitian ini bertujuan untuk mengklon dan mengkarakterisasi gen pengkode a-amilase yang ditapis dari DNA metagenom yang diisolasi dari air dan sedimen laut-dalam perairan Pulau Kawio, Sulawesi Utara. DNA metagenom berhasil diisolasi dari sampel air dan sedimen laut-dalam dan dengan menggunakan teknik *Whole Genome Amplification* (WGA) berhasil ditingkatkan kuantitasnya hingga mencapai 146,31 ng untuk setiap 1 ng DNA metagenom. Suatu fragmen parsial berukuran ~1000 pb berhasil diisolasi dengan menggunakan metode *touchdown PCR*. Fenomena kehadiran transposon dalam fragmen parsial tersebut terungkap pada saat proses kloning di mana terjadi perubahan ukuran fragmen menjadi hanya 310 pb pada akhir tahapan kloning. Analisis hasil sekuensing menunjukkan kemiripan tertinggi fragmen tersebut dengan gen pengkode *replication protein* (Rep) dari bakteri *Pseudomonas putida* (Query Coverage: 88%; Max. Identity: 80%, Positive: 86%) dan protein ini diketahui terlibat pada proses replikasi plasmid di mana gen pengkode transposase juga diketahui hadir bersama gen ini (gen Rep) pada plasmid suatu bakteri.

Kata Kunci: transposon, metagenomik, fragmen parsial, kloning, laut dalam

Permalink/DOI: http://dx.doi.org/10.15578/squalen.v8i3.84

Copyright © 2013, Squalen BMFPB, ISSN 2089-5690

1. Introduction

The marine environment is a landscape that has unique physical, chemical, and biological characteristics. From the point of the study of biology, deep-sea environments, including hydrothermal vent ecosystems, has its own uniqueness. Unique characteristics of enzymes and secondary metabolites produced by marine microorganisms-in to benefit the search of new enzymes (novel enzymes) that are useful for industrial purposes, food, and raw materials of drugs (Steele et al., 2005). One of the interesting enzymes is α-amylase, an enzyme that has wide scale use in biotechnology-based industries, especially in starch-based food industries to convert polysaccharide into simple sugars such as glucose, maltose, dextrin, and other oligosaccharides (Rahmani et al., 2011).

Metagenomic is an approach in genomic analysis to gain insight into the genetics of entire microbial communities without cultivation process of certain microbial species (Schloss & Handelsman, 2003). Since it is known that only about 1% of microbes that can be cultured (culturable) and the remaining, more than 98% are microbial communities that cannot be cultured (unculturable) (Sharma, 2005).then metagenomic offer another approach that can access a large number of microbes that cannot be cultured (Handelsman et al., 2007). The exploration of marine microbial community through metagenomic approach initiated in 1985, when Pace and his team conducted a total DNA extraction from marine plankton to obtain genetic information from its ligated fragments (Lane et al., 1985; Vakhlu et al., 2008).

Transposable elements are DNA sequences that are capable of mediating their own movement (transposition) to new locations within the genome they inhabit. In bacteria, transposable elements can generally be assigned to one of two major types, "Insertion Sequences (IS)" and "Composite Transposons". In practice, composite transposons are typically referred to simply as "transposons". Insertion sequences (IS's) are transposable elements whose only have genes that are directly related to promotion and regulation of their transposition, typically the gene is called as a transposase gene. IS elements are between 700 - 2,000 bp in length and are characterized by short, terminal, inverted repeat sequences with the ORF or ORF's in between. They are normal constituents of many bacterial chromosomes and plasmids. Composite transposons generally consist of two copies of the same IS element flanking variable amounts of other DNA sequences coding for one or several genes with diverse functions. The best known transposons are those which were discovered as parts of antibiotic resistance plasmids (Aziz et al., 2010).

Several enzymes have been successfully explored from deep-sea environment through metagenomic approach include: xylanase (Collins et al., 2002), protease (Zeng et al., 2003), chitinase (Hobel et al., 2005), esterase (Ferrer et al., 2005; Park et al., 2007), amylase (Mathur et al., 2006), alkaline hydroxylase (Xu et al., 2008), and lipase (Jeon et al., 2009). In the initial, this research was aimed to clone and characterize the gene encoding α–amylase from metagenom DNA derived from sea-water and sediment in the waters of West Kawio island, North Sulawesi.

2. Materials and Methods

2.1. Deep-sea Water and Sediment

The deep-sea water and sediment that used as main samples of this research were obtained from 1500-3000 m depth bellow the sea surface of West Kawio Island, Sangihe Talaud archipelago, North Sulawesi where the point of sampling station located at 5° 722 03 N dan 127° 142 03 E. Generally, physical and chemical condition of sampling area were described as high pressure with 317 atm; the temperature surrounding the vent was measured in range of 35-80°C; pH in range of 2,8-6,5; and salinity in range of 35-40 ppt. This samples were collected (with permission) by The INDEX-SATAL ocean expedition that was conducted on July-August 2010 as international collaboration between the government of Indonesia and The United State of America.

2.2. Metagenomic DNA isolation and Whole Genome Amplification (WGA)

Metagenomic DNA was isolated from 300 ml of deep-sea water using Rapid Water™ Kit (MOBIO®) and its quantities were increased through *Whole Genome Amplification* (WGA), a method based on *Multiple Displacement Amplification* (MDA), using Repli-G Mini Kit™ (QIAGEN®).

2.3. Screening of α-Amylase Encoding Gene

Screening of α-amylase encoding gene was done through *touch-down* PCR method using PCR 2G Robust Hotstart™ (KAPA®). The PCR component consist of Buffer A (1X), $MgCl_2$ (0,5 mM), dNTP mix (0,2 mM), Primers (0,1 ìM), KAPA 2G Robust Polymerase (5 u), DNA template (100 ng), and Nuclease free water (up to 10 ìL final volume). The PCR primers were designed from a consensus of 17 α-amylase encoding genes databases of several marine bacterias. These primers sequences are described as 5'-CATATGCGATTT GAA AGG CGTCA CGGCG-3' (*forward primer*) and 5'-GGAT

Copyright © 2013, Squalen BMFPB, ISSN 2089-5690

CCACATAGACGCTGACAATCGCCGCA-3' (*reverse primer*). The PCR was run on 47-57 °C annealing temperature range with products were estimated 1281 bp in length.

2.4. Cloning of α-Amylase Encoding Gene

The α-amylase encoding gene was cloned using pGEM™-T Easy cloning vector (PROMEGA®). The cloning was done within *E. coli* DH5α competent as the host cell, that has been optimized into 1,96 x 10^6 cfu μg of plasmid DNA as its transformation efficiency (Tu et al., 2005; Roychoudhury et al., 2009).

2.5. Sequencing Analysis

Sequencing service was done by Macrogen, Inc. (Korea). EXPASY Translate Tool™ *(http://web.expasy.org/translate/)* was used to translate the consensus of nucleotide sequences into amino acid sequences and also to predict *open reading frame* (ORF) within the sequences. Further bioinformatics analysis of the chosen ORF were done to characterize conserved domains, active sites, and protein motifs of the sequence.

3. Results and Discussion

3.1. Metagenomic DNA Isolation and WGA

Isolation was successfully obtained ~21 kb of metagenomic DNA in length as 104 ng (in 100 μl) in quantities. Through WGA, it could be increased in quantities to 146,31 ng for each 1 ng of metagenomic DNA (Figure 1).

WGA was done using *multiple displacement amplification* (MDA) mechanism, utilized the unique activities of phi29 DNA polymerase and *degenerate hexamer* primers within *isothermal* temperature at 30°C (Dean et al., 2002). Phi29 DNA polymerase is from Õ29 bacteriophage and it was proved able to add ~70.000 of nucleotide (dNTPs) for every single primers bind, and it made MDA could synthesis long product of amplification, up to 200 kb. It was important for genomic library construction, with less of error proofed when compared with conventional PCR method (Lasken et al., 2003) (Paez et al., 2004).

3.2. Screening of α-Amylase Encoding Gene

Screening of α-amylase encoding gene was done through *touchdown* PCR method on 47-57°C annealing temperature range. As the result, there were multiple bands appeared in gel electrophoresis (Figure 2). The single band at ~1000 bp in length seemed thicker than others. This band then be purified to be cloned.

3.3. Cloning of α-Amylase Encoding Gene

DNA fragment of ~1000 bp that has been purified from gel electrophoresis then be ligated to pGEM™-T Easy cloning vector (PROMEGA®). Competent cells of *E. coli* DH5α then be transformed by the ligated plasmids. In this research we have done for four times of transformation. For each transformation, plasmids of positive clones then be isolated. At the first transformation, there were 7 positive clones that successfully being isolated its plasmids. The confirmation of *uncut* plasmid within these clones has been done using electrophoresis (Figure 3).The observation of *uncut* plasmids migration on gel electrophoresis showed that the seven positive clone plasmids have similar migration distance with circular *uncut* of pGEM™-T Easy (no insert DNA). It showed that the insert DNA of the positive clone plasmids have just short in length. The plasmids might that migrate over the pGEM™-T Easy were guessed containing an insert DNA. Confirmation of these insert was done using PCR method (Figure 4).

Figure 4 told us that all of 5 positive clone plasmids that have been confirmed using first early primers resulted a ~300 bp band product as insert, not ~1000 bp as initial insert during ligation.

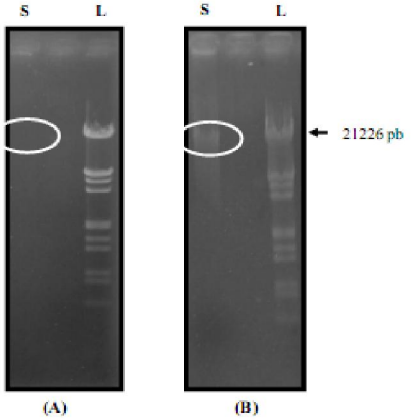

Figure 1. Electroferogram (A) Result of Isolation of Metagenomic DNA, (B) Result of *Whole Genome Amplification* (WGA); L : DNA λ Hind III + EcoR1 Ladder (FERMENTAS®); S : Sample.

Copyright © 2013, Squalen BMFPB, ISSN 2089-5690

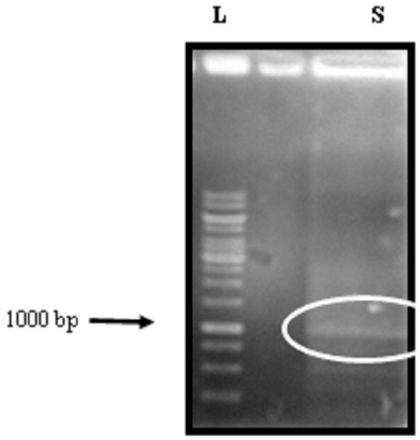

Figure 2. Result of Touchdown PCR; L: 1 kb DNA Ladder™ (FERMENTAS®) ; S: Sample.

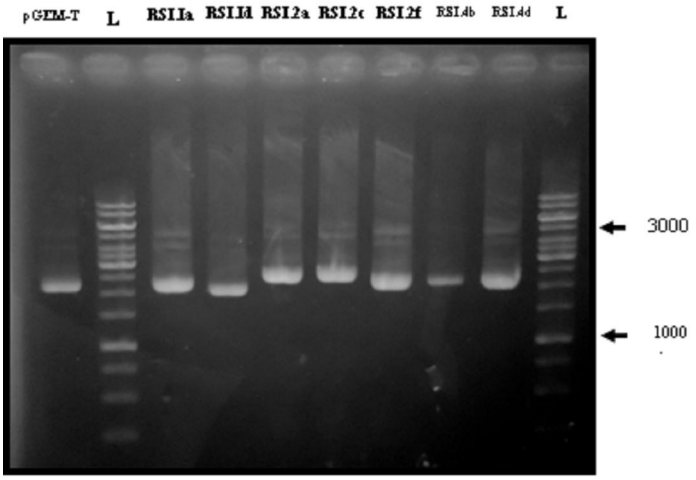

Figure 3. Electroferogram of *uncut* plasmid migration of isolated positive clone plasmids at 1st transformation; L: 1 kb DNA Ladder™ (FERMENTAS®); RSI.[1,2,3,4].[a,b,c,…..etc] : Plasmids of Positive Clones

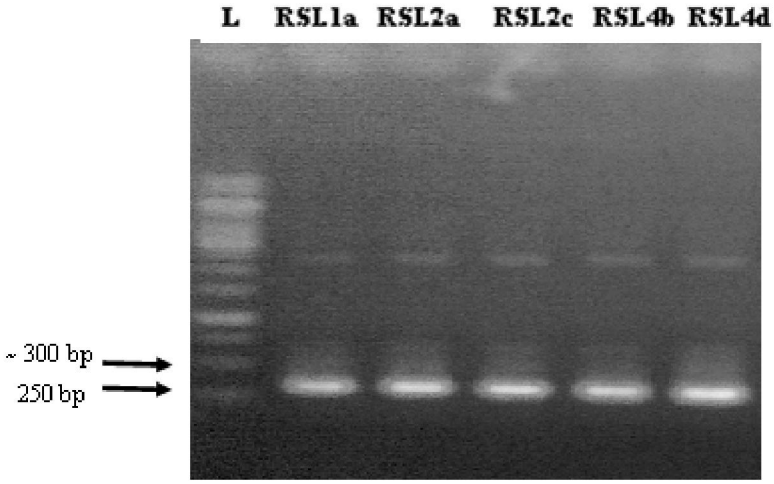

Figure 4. Electroferogram of Insert DNA confirmation based on PCR method L: 1 kb DNA Ladder™ (FERMENTAS®) RSI.[1a; 2a; 2c; 4b; 4d] : Plasmids of positive clones

Copyright © 2013, Squalen BMFPB, ISSN 2089-5690

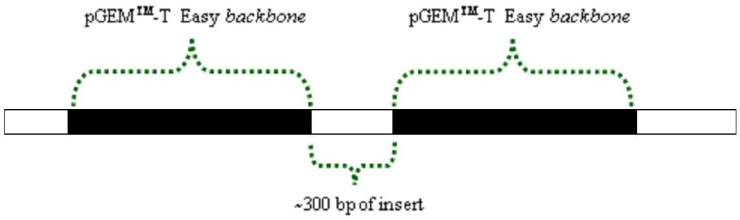

Figure 5. VecScreen™ Output of Sequencing Consensus Sequence.

3.4. Sequencing Analysis

The plasmid containing insert then was sent for sequencing. It was done using T7 (*forward*) and SP6 (*reverse*) primer pairs. Analysis of the result using VecScreen™ *(http://www.ncbi.nlm.nih.gov/VecScreen)* showed that only ~300 bp insert DNA that has been ligated into pGEM™-T Easy vector cloning (Figure 5).

Further analysis of this insert sequence also showed that sequence of primers were found flanked the insert sequence (Figure 6). Thus, the change of insert sequence length from ~1000 bp at initial ligation became 310 bp in the end of cloning must be clarified for several reasons. The first hypothesis be proposed correlated with homolog recombination by host cell. But this hypothesis was failed since *E. coli* DH5α has *recA1* in its genotyping. This mutation gene prevented the host to do a homolog recombinant to its contained plasmid (Hanahan et al., 1985). The same pattern of fragment size for every transformation confirmation (always produced ~300 bp partial fragment) also seemed very rare for homolog recombination activity.

The second hypothesis that been proposed to clarify the change of insert sequence during transformation was correlated to transposable DNA activity. In bacteria, transposon (famous name of a transposable DNA) could jump from chromosome to plasmid and its contrary (Berg et al., 1984). One type of transposons in bacteria known as *insertion sequence* (IS); it does not contain any additional genes except a gene coding transposase, an enzyme that correlated to transponsition activities. IS was known as 768 – 1426 bp in length (McClean, 1997). This hypothesis also being proved since it was found a direct repeat sequence GCCATTGCCATT within the 310 bp partial fragment (Figure 7). The structure of an IS consist of a transposase encoding region, flanked by an inverted repeat sequence (9-50 bp) and the 5' and 3' short direct repeats (5-11 bp) that generated from the target-site DNA during the insertion of IS (Calos & Miller, 1980).

The figure below (Figure 8) explained an illustration of hypothesis correlated with transposition activity of an IS (estimated ~700 bp in length) during transformation. At the initial stage of this research (screening of gene), the IS has been estimated still remain linked within the whole screened fragment (we could see the initial size was ~1000 bp), but during the transformation this IS was moved out from the plasmid and left just 310 bp partial fragment in the end of cloning.

As the next stage of the research, we found that the translation of thus 310 bp of partial fragment have 2 strand of possible ORF, they were *frame* 1 (52-32) as 67 aa in length and *frame* 3 (32-52) as 103 aa (Figure 9). This phenomena was similar with a concept of overlapping gene. The overlapping gene also found in bacteria, since it was abundant in virus (Ellis & Brown, 2003).

From table 1, we knew that BLASTp result of ORF at *frame* 1 (52-32) with nr protein database showed a similarity with *Glycogen Debranching Enzyme* GlgX [*Actinomyces* sp. oral taxon 448 str. F0400] with *query coverage* = 56%, *max. identity* = 39%, and *positive* = 57% (NCBI; (Muzny et al., 2011)). *Glycogen debranching enzyme* GlgX was include as glycoside-hydrolase group (GH) family 13, as the same family as α-Amylase, α-Amylase, Glucan 1,4-α-glucosidase, etc (http://enzyme.expasy.org/EC/3.2.1.-). In the deep water ocean it was very rare to find polysaccharides

Copyright © 2013, Squalen BMFPB, ISSN 2089-5690

CATATGCGATTTGAAAGGCGTCACGGCGTACTTGAACACTTCCATGAAGCCCTCAACGGGATCACCCAGGATGGGCCTGCAA
TCCACGACCATGCTGTCACCGGTGATCTTATGCCACTCGGCGCTAAGGTCAGTTTGGGATGGCTGAGAGGTAGCCAGAATGA
TCATGTGGCAGTGCGGATGCCAGCCATTGCCATTGTTGGTTAGCTCAAGGGTGTAAACCGCTCCCTCTGCCTTGCAAAGCTC
TGTCCATGGAGCCCGAGGAACGCCGGAATTGAAGTTGCGGCGATTGTCAGCGTCTATGTGGATCC

Note :

GCCATTGCCATT : Direct repeat sequence

Figure 7. Direct repeat sequence within the 310 bp insert sequence.

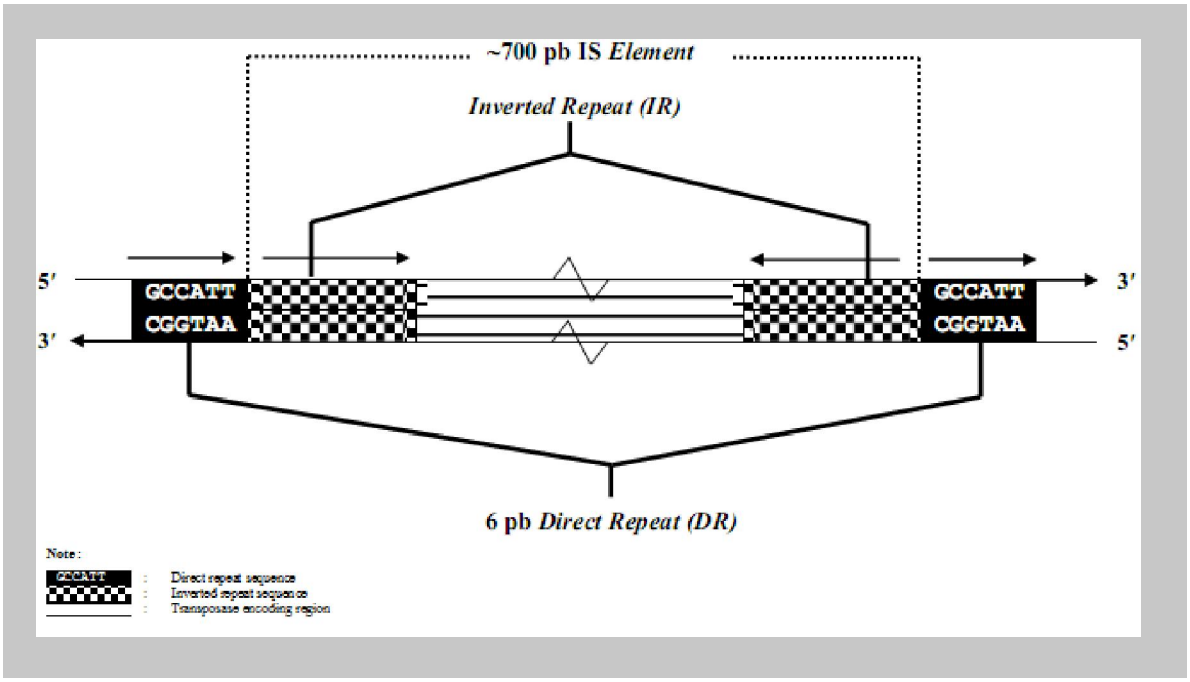

Figure 8. An illustration of Transposons Hypothesis.

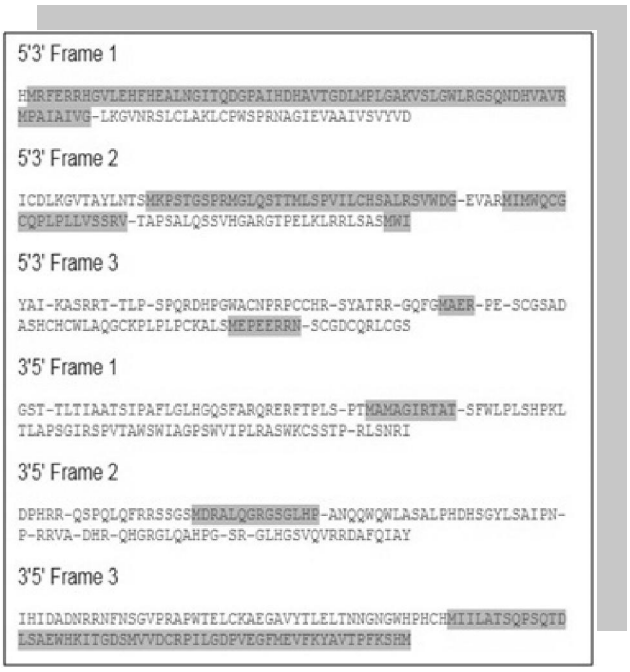

Figure 9. Translation and ORF prediction : Indicate ORF.

Copyright © 2013, Squalen BMFPB, ISSN 2089-5690

Table 1. BLASTp Result Summary between ORF to Protein "nr" dan "env_nr" Databases

No	Database Name	BLASTp Result	Query Coverage	Max. Identity	Positive
ORF 5'-3' Frame 1 (67 aa)					
1	nr	putative aminotransferase [*Streptomyces parvulus*]	65%	43%	54%
		aldehyde dehydrogenase [*gamma proteobacterium* HdN1]	80%	31%	58%
		phosphorylase kinase alphabeta [*Halothiobacillus neapolitanus* c2]	77%	42%	54%
		GntR family transcriptional regulator [*Pseudomonas* sp. TJI-51]	83%	34%	46%
		glycogen debranching enzyme GlgX [*Actinomyces* sp. oral taxon 448 str. F0400]	56%	39%	57%
2	env_nr	hypothetical protein GOS_7923894 [marine metagenome]	55%	35%	51%
ORF 3'-5' Frame 3 (103 aa)					
1	nr	replication protein [*Pseudomonas putida*]	88%	80%	86%
		unnamed protein product [*Nitrosomonas sp*.]	87%	57%	71%
		RepA [*Methylophaga thalassica*]	87%	48%	61%
		hypothetical protein, partial [*Acinetobacter* sp. NIPH 236]	86%	47%	53%
		replication protein Rep [uncultured bacterium]	66%	51%	64%
2	env_nr	hypothetical protein GOS_2869860 [marine metagenome]	87%	46%	57%
		hypothetical protein GOS_3844660 [marine metagenome]	30%	55%	80%
		hypothetical protein GOS_2712569 [marine metagenome]	45%	40%	55%
		hypothetical protein GOS_9602977 [marine metagenome]	66%	28%	44%
		hypothetical protein GOS_7442391 [marine metagenome]	70%	30%	45%

as substrate, then several starch hydrolyzing-enzymes might used glycogen as its substrate (Leveque et al., 2000).

Table 1 also showed that from inverse strand that was *frame* 3 (32–52), BLASTp result indicated a similarity between query sequence to a *replication protein* of *Pseudomonas putida* with *query coverage* = 88%, *max. identity* = 80%, and *positive* = 86% (NCBI; (Holtwick et al., 2001)). *Replication protein* (Rep) was a protein encoding by a plasmid and was known involved in plasmid replication process. *Rep* encoding gene mostly found in plasmids that used *theta* mechanism as its replication mechanism, even though it was also found in any plasmids that used

Copyright © 2013, Squalen BMFPB, ISSN 2089-5690

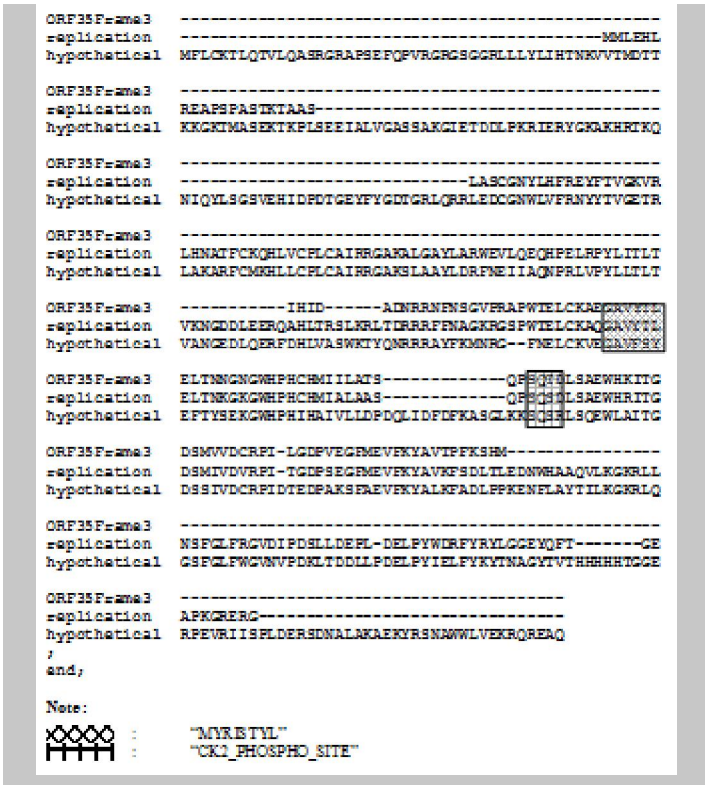

Figure 10. Multiple alignment result of Sekuen ORF frame 3 (32-52), *Replication Protein* [*Pseudomonas putida*], and *Hypothetical Protein*_GOS2869860.

both *strand displacement replication* and *rolling-circle replication* during replication process (Solar G et al., 1998).

When used "env_nr", BLASTp result showed that the ORF at *frame* 3 (32–52) also similar with *hypothetical protein* GOS-_2869860 (Query Coverage: 87%; Max. Identity: 46%, Positive: 57%). *Hypothetical protein*_GOS databases were collected during expedition of "*The Sorcerer II Global Ocean Sampling (GOS) Expedition : Northwest Atlantic through Eastern Tropical Pacific*" on 2003 and 2004 (Yooseph et al., 2007). This expedition was sponsored by *The J. Craig Venter Institute* in order to explore genome databases form marine bioresources (Rusch et al., 2007).

3.5 Conserved Domain, Active Site and Protein Motif Characterization

Further characterization was done for ORF at *frame* 3 (32 – 52), since it has the highest value of BLASTp result. In this stage, the ORF also being compared with *replication protein* sequence from *Pseudomonas putida* and *hypothetical protein* GOS_2869860 sequence from marine metagenomic research.

Conserved domain analysis using *Conserved Domian Database* (cdd®) (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) showed that Rep_1_superfamily" domain was found as conserved domain of this ORF, this domain also found in both *replication protein* sequence from *Pseudomonas putida* and *hypothetical protein* GOS_2869860 sequence from marine metagenomic. Rep_1_superfamily" domain indicated region of replication protein encoding, and known that it was involved in plasmid replication process (Marsin et al., 1998). By using EXPASY Prosite™ (http://prosite.expasy.org/), active sites of ORF sequence were identified. But the result showed that there were no active site found in the ORF sequence, also in both *replication protein* sequence from *Pseudomonas putida* and *hypothetical protein* GOS_2869860 sequence from marine metagenomic. Protein motifs of each sequence were identified using MotifScan™ (http://myhits.isb-sib.ch/cgi-bin/motif_scan). The result showed there were 3 protein motifs found in the ORF sequence, they were "*MYRISTYL*" was found at 28-33 [GAVYTL], "*CK2_PHOSPHO_SITE*" was found at 57-60 [SQTD], and "MICROBODIES_CTER" [SHM] was found at 101-103. The result also showed that the ORF has similarity with *replication protein* sequence from *Pseudomonas putida* and *hypothetical protein* GOS_2869860 sequence from marine metagenomic in the motifs of "MYRISTYL" and "CK2_PHOSPHOP_SITE" (Figure 10).

Copyright © 2013, Squalen BMFPB, ISSN 2089-5690

4. Conclusion

The α-amylase encoding gene could not isolated from metagenomic DNA derived from deep-sea water and sediment in this research. But, a fragment as ~1000 bp in length was successfully isolated through *touchdown* PCR method. The presence of a transposon in this DNA fragment is proposed as a hypothesis for losing ~700 bp leaving just 310 bp cloned sequence. This hypothesis was supported by the presence of a direct repeat sequence GCCATTGCCATT within the 310 bp partial fragment that indicated a transposon trait. Analysis of sequencing result showed a highest similarity between this 310 bp partial fragment with a replication protein (Rep) encoded gene from Pseudomonas putida (Query Coverage: 88%; Max. Identity: 80%, Positive: 86%) and this protein is known to be involved in plasmid replication.

5. Acknowledgement

This research was supported by grant from Hibah Riset KK SITH-ITB 2010/2011.

Reference

Aziz, R. K. M., Breitbart, & Edwards, R. A. (2010). Transposases are the most abundant, most ubiquitous genes in nature. *Nucleic Acids Research*, *38*(13), 4207–4217.

Berg, D. E., Berg, C. M., & Sasakawa C. (1984). Bacterial Transposon Tn5: Evolutionary Inferences. *Mol. Biol. Evol., 1*(5), 411-422.

Calos, M. P. & Miller, J. H. (1980). *Cell.* p. 1280.

Collins, T., Meuwis, M. A., Stals, I., Claeyssens, M., Feller, G., & Gerday, C. (2002). A novel family 8 xylanase, functional & physicochemical characterization. *J. Biol. Chem., 277*, 35133–35139.

Dean, F. B., Hosono, S., Fang, L., Wu, X., Faruqi, A.F., Bray-Ward, P., Sun, Z., Zong, Q., Du, Y., Du, J., Driscoll, M., Song, W., Kingsmore, S. F., & Egholm, M. (2002). *Comprehensive Human Genome Amplification using Multiple Displacement Amplification*. Proceedings of The National Academy of Sciences. USA 99: 5261-5266.

Ferrer, M., Golyshina, O. V., Chernikova, T. N., Khachane, A. N., Martins Dos Santos, V. A., Yakimov, M.M., Timmis, K.N., & Golyshin, P.N. (2005). Microbial enzymes mined from the Urania deep-sea hypersaline anoxic basin. *Chem. Biol., 12,* 895–904.

Hanahan, D. (1985). *In DNA Cloning: A Practical Approach* (Glover, D.M., ed.)(p. 109), Vol. 1, McLean, Virginia: IRL Press.

Handelsman, J., James, M., Tiedje, Cohen. L. A., Michael Ashburner, ... Ann, H. R (p. 24). (2007). *Understanding Our Microbial Planet: The New Science of Metagenomics.* The National Academy of Science.

Hobel, C. F., Hreggvidsson, G. O., Marteinsson, V. T., Bahrani-Mougeot, F., Einarsson, J. M., & Kristjansson, J.K. (2005). Cloning, expression, & characterization of a highly thermostable family 18 chitinase from Rhodothermus marinus. *Extremophiles*, *9,* 53–64.

Jeon, J. H., Kim, J. T., Kim, Y. J., Kim, H. K., Lee, H. S., Kang, S. G., Kim, S. J., & Lee, J.H. (2009). Cloning & characterization of a new cold-active lipase from a deep-sea sediment metagenome. *Appl. Microbiol. Biotechnol.*, *81*, 865–874.

Lane, D. J., Pace Bernadettl, Olsen G. J., Stahl, D. A., Sogin, M. L., & Pace, N. R. (1985). *Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl. Acad. Sci., 82,* 6955–6959.

Lasken R. S. & Egholm, M. (2003). Whole-genome ampliûcation: abundant supplies of DNA from precious samples or clinical specimens. *Trends Biotechnol., 21,* 531.

Mathur, E. J., Toledo, G., Green, B. D., Podar, M., Richardson, T. H., Kulwiec, M., & Chang, H. W. (2006). A biodiversity-based approach to development of performance enzymes. *Ind. Biotechnol., 1*, 283–287,

McClean, P. (1997). Bacterial Transposable Element. *Review of Transposable Genetics Element. 2,* 11-13.

Paez, J. G., Lin, M., Beroukhim, R. Lee, J. C, Zhao, X., Richter, D. J., Gabriel, S., Herman, P., Sasaki, H., Altshuler, D., Li, C., Meyerson, M., & Sellers, W. R. (2004). Genome coverage and sequence fidelity of the phi29 polymerase-based multiple strand displacement whole genome ampliûcation. *Nucleic Acids Research, 32,* 71.

Park, H. J., Jeon, J. H., Kang, S. G., Lee, J. H., Lee, S. A.; & Kim, H. K. (2007). Functional expression and refolding of new alkaline esterase, EM2L8 from deep-sea sediment metagenome. *Protein Expr. Purif., 52,*: 340–347.

Rahmani, Nanik, Yopi, Andriani, A., & Prima, A. (2011). Production and Characterization of Amylase Enzyme from Marine Bacteria. *Proceedings of the 2nd International Seminar on Chemistry.* pp. 255-259.

Roychoudhury, A., Basu, S., & Dibyendu, N. S. (2009). Analysis of Comparative Efficiencies of Different Transformation Methods of E. coli using Two Common Plasmid Vectors. *Indian Journal of Biochemistry and Biophysics, 46,* 395-400.

Schloss, P. D. & Handelsman, J. (2003). Biotechnoligical prospects from metagenomics. *Curr. Opin. Biotechnol., 14*, 303–310.

Sharma, R. (2005). *Unculturable' bacterial diversity. An untapped resource. Curr. Sci., 89,* 72–77.

Steele HL and Streit WR. (2005). Metagenomics: advances in ecology and biotechnology. *FEMS Microbiol. Lett.,* 247, 105–111.

Tu, Z., He, G., Li, K. X., Chen, M. J., Chang, J., Chen, L., Yao, Q., Liu, D. P., Ye, H., Shi, J., and Wu, X. (2005). An Improved System for Competent Cell Preparation & High Efficiency Plasmid Transformation using Different Escherchia coli Strains. *Electronic Journal of Biotechnology, 8*(1), 113-120.

Vakhlu, J., Sudan, A. K., & Johri, B. N. (2008). Metagenomic : Future of microbial gene mining. *Indian J. Microbiol., 48*, 202-215.

Xu, M., Xiao, X., & Wang, F. (2008). *Isolation & characterization of alkane hydroxylases from a metagenomic library of Pacific deep-sea sediment*. Extremophiles, *12*, 255–262.

Zeng, R., Zhang, R., Zhao, J., & Lin, N. (2003). Cold-active serine alkaline protease from the psychrophilic bacterium Pseudomonas strain DY-A: enzyme purification & characterization. *Extremophiles, 7,* 335–337.

Copyright © 2013, Squalen BMFPB, ISSN 2089-5690