MICROBIOLOGY
INDONESIA
ISSN 1978-3477, eISSN 2087-8575
Vol.13, No.4, December 2019, p 123-128

Available online at
http://jurnal.permi.or.id/index.php/mionline
DOI: 10.5454/mi.13.4.3

# Gene Cloning of Xylanase Glycoside Hydrolase Family 11 from *Bacillus halodurans* CM1 in *Escherichia coli* DH5α

MUHAMAD TAUFIQUL NAUFAL[1], AGUSTIN KRISNA WARDANI[1], AND IS HELIANTI[2]*

[1]*Faculty of Agricultural Technology, Universitas Brawijaya,*
*Jalan Veteran, Ketawanggede, Lowok Waru, Malang 65145, East Java, Indonesia;*
[2]*Center for Bioindustrial Technology, (Agency for Assessment and Application Technology BPPT), 614 Building, LAPTIAB-BPPT, Puspiptek-Serpong, South Tangerang, Banten, Indonesia.*

Xylanase is an enzyme that can break down xylan into xylose and xylooligosaccharide that is widely used in industry. Because of many applications of this enzyme, researchers conducted many studies on how to increase the productivity of the xylanase enzyme. One of the method that can be used to increase the xylanase enzyme productivity is by using recombinant DNA technology such as cloning. *Bacillus halodurans* CM1 is a local alkalothermophilic bacterium that potential producer for xylanase and other industrial enzymes. The aim of this research is to conduct the cloning of GH11 xylanase coding gene from *Bacillus halodurans* CM1 using pJET 1.2 / blunt plasmid vector into *Escherichia coli* DH5α as cell host and determine the sequence of the GH11 xylanase coding gene from this strain. The results showed the GH11 xylanase gene from *Bacillus halodurans* CM1 was successfully cloned in *Esherichia coli* DH5α and based on the results of BLAST nucleotides had 99% similarities with that of endo-1,4-beta -xylanhydrolase (xyn11A) from other *Bacillus halodurans* C-125.

Key words: *Bacillus halodurans* CM1, cloning, xylanase glycoside hydrolase family 11

Xilanase adalah enzim yang dapat memecah xilan menjadi xilosa dan xilooligosakarida dan banyak digunakan dalam industri. Melihat banyaknya aplikasi enzim ini, para peneliti melakukan banyak studi tentang cara meningkatkan produktivitas xilanase. Salah satu metode yang dapat digunakan untuk meningkatkan proses produksi xilanase adalah dengan menggunakan teknologi DNA rekombinan seperti kloning. *Bacillus halodurans* CM1 adalah galur bakteri asli Indonesia yang bersifat alkalothermophilic yang merupakan penghasil potensial xilanase maupun enzim industri lainnya. Penelitian ini bertujuan untuk mengkloning gen pengkode xilanase glycoside hydrolase family 11 (GH11) dari *Bacillus halodurans* CM1 menggunakan pJET 1.2 / blunt plasmid vektor ke dalam *Escherichia coli* DH5α sebagai sel inang dan menentukan urutan basa dari gen pengkodean xylanase GH11 dari galur ini. Hasil penelitian menunjukkan gen xylanase GH11 dari *Bacillus halodurans* CM1 berhasil dikloning di *Esherichia coli* DH5α, dan berdasarkan hasil analisa BLAST deret DNA menunjukkan kemiripan dengan deret DNA endo-1,4-beta -xylanhydrolase (xyn11A) dari *Bacillus halodurans* C-125 sebesar 99%.

Kata kunci: kloning, *Bacillus halodurans*, xilanase *glycoside hydrolase family* 11

Xylanase is an enzyme that can break down xylan into xylose and xylooligosaccharides, and has been used in various industries such as paper, animal feed, beverage , bread, and pharmaceutical, etc. Xylanase can be used in the process of pulp bleaching so it can reduce the use of chlorine. In animal feed industry, xylanase was used as an enhancer for the digestibility of animal feed derived from cereals. In addition, xylanase is also used to produce prebiotics like xylooligosaccharides that can be used in the pharmaceutical industry. Seeing many applications of this enzyme, many researchers conducted study on how to increase the productivity and effectiveness xylanase so the economy feasibility of xylanase enzyme production can be improved (Shahi *et al.* 2016). Improvement of xylanase can be conducted by isolation of a new type of enzyme that more effective in application, protein engineering, and recombinant DNA technology (Singh *et al.* 2016).

Recombinant DNA technology is used to change the genetic material of an organism so new organisms can be obtained with the desired characteristics. Recombinant DNA technology is carried out by inserting target DNA fragments into an organism or host with the help of a vector. One type of activity on recombinant DNA technology is cloning (Khan *et al.* 2016). Cloning is a procedure for isolating a particular DNA sequence and multiplying that sequence in a cloning vector (Buwono *et al.* 2018). Gene cloning can isolate the specific DNA sequences that encode desired enzymes and study the components that affect the performance of the DNA sequences such as regulation of gene expression, compatibility with host cells and

*Corresponding author: Phone: +62-21-217650; Email: is.helianti@bppt.go.id

vectors, and the addition of certain promoters.

Cloning of xylanase genes from various sources of microorganisms has been carried out in various host cells. Sriyapai *et al* (2011) have cloned xylanase which has heat resistance from *Actinomadura* sp S14 and expressed it on *Escherichia coli* and *Pichia pastoris*. Jeya *et al* (2009) have reported research on cloning and expression of GH11 xylanase from *Aspergillus fumigatus* MKU1 in *Pichia pastoris*. Cloning and characterization of xylanase-coding genes from the isolate *Bacillus subtilis* R5 strain in *Escherichia coli* have been reported by Jalal *et al* (2009). Alkalothermophilic bacteria such as *Bacillus halodurans* CM1 is a potential source of xylanase-coding genes because that microorganism live in extreme environments (Ulfah *et al.* 2011).

*Bacillus halodurans* CM1 is an alkalothermophilic bacteria isolated from Cimanggu hot springs in West Java which is known to be able to produce xylanases with optimum activity at pH 9 and 70 °C. From the genetic information of *Bacillus halodurans* C-125, whose whole genome information available, has three types of xylanases which have different sizes, with 45, 30, and 20 kDa. Research on cloning of xylanase-coding genes from *Bacillus halodurans* CM1 which has a size of 30 kDa has been conducted by Safirah et al (2016) and research on cloning of xylanase-coding genes from *Bacillus halodurans* CM1 bacteria which has a size of 45 kDA has also been done by Helianti et al (2018). However, research on cloning of xylanase-coding genes from *Bacillus halodurans* CM1 which has size of 20 kDA which is considered to be a GH11 xylanase has never been done before. Therefore, it is necessary to clone the xylanase coding gene from the bacterium *Bacillus halodurans* CM1 which has a size of 20 kDA which is predicted to be a GH11 xylanase based on the GH11 xylanase sequence from *Bacillus halodurans* C-125. In this work amplification of xylanase GH11 gene using designed primer based on data base on Genbank, cloning into *E. coli* DH5 α, and analyses of the sequenced DNA are described.

## MATERIALS AND METHODS

**Reagent, Plasmid, Media, and Bacterial Strains.** The following materials and chemicals were used: Etilen diamin tetra asetat (EDTA), T4 DNA ligase, Go Taq Flexi DNA Polymerase (Promega), MyTaqHSRedmix (Bioline), tryptone (Biomatik), yeast extract (Himedia), NaCl, agarose (Thermo Scientific), Ampicilin, $ddH_2O$, buffer tris acetate EDTA (TAE) 1X, tris buffer fenol, buffer Tris EDTA (TE), natrium acetat, etidium bromida (EtBr), isopropranol, TB (tranformation buffer), dimetil sulfoxida (DMSO), etanol 70%, loading dye, DNA ladder 1 kb (Fermentas), primer Fwd-ORF-xyn11 dan Xyn11-Bhal-rev, restriction enzyme BglII (New England Biolab), Gel/PCR DNA Extraction Kit (Geneaid). CloneJET PCR Cloning Kit (Thermo Scientific). Horikoshi media, luria bertani media, super optimal broth (SOB) cair, super optimal broth with catabolite repressien (SOC), *Bacillus halodurans* CM1, and *Escherichia coli* DH5α.

**Genomic DNA Extraction of *Bacillus halodurans* CM1.** Genomic DNA from *Bacillus halodurans* CM1 was isolated by the method of phenol-chloroform DNA extraction (Saito dan Miura, 1963). The result of extraction genome visualisation was observed in agarose 1% by electrophoresis.

**Amplification of Xylanase GH11 Gene.** Xylanase GH11 gene was amplified using GoTaq® Flexi DNA Polymerase (Promega) and the primers specific for the coding region of xylanase GH11. Primers were designed according to the published sequence of *Bacillus halodurans* C-125. Primer sequences were as following: the forward primer was Fwd-Orf-Xyn11: 5'-ATG TTT AAG TTC GTT ACG AAA G -3' and the reverse primer was Xyn11-Bha1-rev: 5'- CTA CCA AAC CGT CAC ATT CGA -3'. PCR reactions were performed in a total volume of 50 µl containing 1X GoTaq Flexi Buffer, 3mM MgCl2, 0.2mM each dNTP, 100 ng of template DNA, 1µM of each primers, 1.25 U of Go Taq Flexi DNA Polymerase, and 29.75 µl of $ddH_2O$. The following conditions were applied: initial denaturation of PCR product was at 95 °C for 1 min, followed by 30 cycles; denaturation at 95 °C for 30 sec, annealing at 52 °C for 30 sec, elongation at 72 °C for 30 sec. The program was followed by final elongation at 72 °C for 5 min. The PCR-amplified products were detected with1% ethidium bromide (EtBr). After electrophoresis, images were obtained in UV gel documentation systems (UK).

**Cloning and Sequencing of the Xylanase GH11 Gene of *Bacillus halodurans* CM1.** First, the PCR product were purified using a Gel/PCR DNA Extraction Kit (Geneaid) according to the manufacturer's protocol. Then, purified products were cloned with CloneJET PCR Cloning Kit (Thermo Scientific) using a blunt cloning technique. pJET1.2/blunt plasmid that contained xylanase GH11 gene was transformed into competent cell *Escherichia*

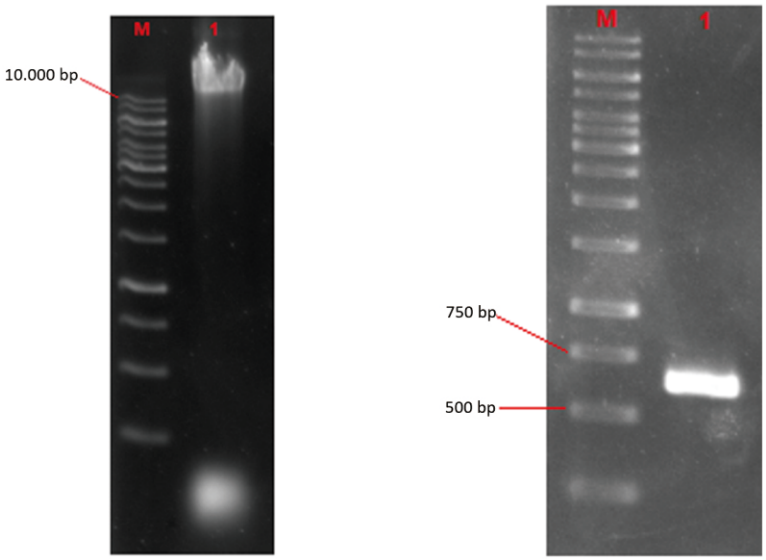


Fig 1 Agarose gel electrophoresis of Genomic DNA extracted from *Bacillus halodurans* CM1 (Lane M is DNA *ladder* 1 kb (Fermentas), Lane 1 is positive samples) (a); PCR amplification of xylanase GH11 gene from B. Halodurans CM1 (b); recombinant plasmid of transformant colony (c); recombinant plasmid from transformant colony (Lane M is DNA *ladder* 1 kb (Fermentas), Lane 1 is positive samples) (d).

*coli* DH5α by heat shock methods (Hanahan 1983). Colony PCR and restriction of plasmid analysis was used to confirm the gene cloning. Xylanase GH11 gene was confirmed from transformant colony by colony transformant using MyTaqHSRedmix (Bioline). Colony PCR reactions were performed in a total volume of 20 µL containing 1X MyTaqHSRedmix, cell of transformant colony, 1µM of each primers, and 8 µL of $ddH_2O$. The program were applied: initial denaturation of PCR product was at 95 °C for 3 min, followed by 30 cycles; denaturation at 95 °C for 15 sec, annealing at 52 °C for 15 sec, elongation at 72 °C for 15 sec. The program was followed by final elongation at 72 °C for 5 min.

Recombinant plasmids from positive transformant were isolated by the method of alkali plasmid extraction (Birnboim dan Doly 1979) and digested with *BglII* restriction enzyme. Restriction reactions were performed in a total volume of 10 µl using manufacturer's protocol. Finally, the sequencing from cloned fragments was done using third party (First Base Asia) and sequence similarity was checked using nucleotide and amino acid BLAST analysis at NCBI.

## RESULTS

Genomic DNA of *Bacillus halodurans* CM1 on 1 % agarose gel revealed a DNA band above the 10.000 bp marker band (Fig. 1a). Analysis of PCR products for the presence of the xylanase GH11 gene of *Bacillus halodurans* CM1 on 1 % agarose gel revealed a ±600-bp fragment (Fig. 1b). After ligation between PCR product and pJET1.2/blunt plasmid, there was only one recombinant colony grew on transformation plate and this colony used for futher analysis. Colony PCR of recombinant colony on 1 % agarose gel revealed a ±600-bp fragment.

Recombinant pJET1.2/blunt plasmid were isolated and revealed a ±3000-bp fragment on 1% agarose gel Restriction analysis of recombinant plasmid of pJET on 1 % agarose gel revealed a ±3000-bp and ±600-bp fragments. The clones that has been confirmed by colony PCR and *BglII* restriction enzyme further used for sequencing. DNA sequencing result showed the nucleotide and amino acid sequence obtained from a fragment of the xylanase GH11 gene of *Bacillus halodurans* CM1 was found to be 99% identical with endo-1,4-beta-xylanhydrolase (xyn11A) gene from *Bacillus halodurans* C-125 (Fig 2 and Fig 3).

## DISCUSSION

Horikoshi medium and 50°C incubation temperature was used for cultivation of *Bacillus halodurans* CM1 beacuse this microorganism was isolated from hot spring which had hight temperature (Ulfah *et al*. 2011). DNA extraction process use organic solvents such as phenol or phenol: chloroform for purification by precipitate the protein (Brown 2010). Polymerase Chain Reaction (PCR) is a technology that allows us to amplify target DNA fragment that is limited by the primer in large quantities

```
   1  ATGTTTAAGTTCGTTACGAAAGTTTTGACGGTAGTAATTGCAGCTACAAT   50
 472  ATGTTTAAGTTCGTTACGAAAGTTTTGACGGTAGTAATTGCAGCTACAAT  521
  51  TAGTTTTTGTTTGAGTGCAGTACCGGCAAGTGCTAATACCTATTGGCAAT  100
 522  TAGTTTTTGTTTGAGTGCAGTACCGGCAAGTGCTAATACCTATTGGCAAT  571
 101  ATTGGACCGATGGTGGTGGAACAGTAAATGCTACAAATGGACCTGGTGGA  150
 572  ATTGGACCGATGGTGGTGGAACAGTAAATGCTACAAATGGACCTGGTGGA  621
 151  AATTACAGTGTGACATGGGGAGATACAGGGAACTTTGTTGTCGGTAAAGG  200
 622  AATTACAGTGTGACATGGAGAGATACAGGGAACTTTGTTGTCGGTAAAGG  671
 201  TTGGGAAATCGGTTCACCAAATCGAACGATCCATTACAATGCTGGTGTTT  250
 672  TTGGGAAATCGGTTCACCAAATCGAACGATCCATTACAATGCTGGTGTTT  721
 251  GGGAACCGTCTGGAAATGGATATTTGACTCTCTATGGGTGGACAAGGAAT  300
 722  GGGAACCGTCTGGAAATGGATATTTGACTCTCTATGGGTGGACAAGGAAT  771
 301  CAGCTCATAGAATATTATGTCGTTGATAATTGGGGAACTTACAGACCTAC  350
 772  CAGCTCATAGAATATTATGTCGTTGATAATTGGGGAACTTACAGACCTAC  821
 351  TGGAACCCATCGAGGCACCGTTGTCAGTGATGGAGGAACATATGACATCT  400
 822  TGGAACCCATCGAGGCACCGTTGTCAGTGATGGGGAACATATGATATCT  871
 401  ATACGACTATGCGATACAATGCACCTTCCATCGATGGCACACAAACGTTC  450
 872  ATACGACTATGCGATACAATGCACCTTCCATTGATGGCACACAAACGTTC  921
 451  CAACAGTTCTGGAGTGTGAGGCAATCGAAGAGACCGACTGGAAATAACGT  500
 922  CAACAGTTCTGGAGTGTGAGGCAATCGAAGAGACCGACTGGAAATAACGT  971
 501  TAGCATTACGTTTAGCAGCCACGTGAATGCGTGGAGAAATGCAGGAATGA  550
 972  TAGCATTACGTTTAGCAACCACGTGAATGCGTGGAGAAATGCAGGAATGA 1021
 551  ATCTGGGAAGTAGTTGGTCTTACCAGGTATTAGCAACAGAAGGCTATCAA  600
1022  ATCTGGGAAGTAGTTGGTCTTACCAGGTATTAGCAACAGAAGGCTATCAA 1071
 601  AGTAGCGGGAGATCGAATGTGACGGTTTGGTAG                   633  CM1
1072  AGTAGCGGGAGATCGAATGTGACGGTTTGGTAG                  1104  C-125
```

Fig 2 Nucleotide BLAST between sequencing result of xylanase GH11 gene from *Bacillus halodurans* CM1 and endo-1,4-beta-xylanhydrolase (xyn11A) gene sequence from *Bacillus halodurans* C-125. The different nucleotide was showed in square.

```
BsubAQ1FJ644630.1          MFKFKKKFLVGLTAAFMSISMFSATASAAGTDYWQNWTDGGGTVNAVNGS
BlichI5ABF61784            MFKFKKNFLVGLTAALMSISLFSATASAASTDYWPNWTDGGGTVNAVNGS
P.catalpaeWP_091185626     MFKFKKKVMTAVLAASMSIGLFAATANAA-TDYWQNWTDGGGTVNAVNGS
P.timonensis_              MMKLKRKMLTLLLTASMSFGLFGATSNAA-TDYWQNWTDGGGTVNAVNGS
CM1                        MFKFVTKVLTVVIAATISFCLSAVPASAN--TYWQYWTDGGGTVNATNGP
                           *:*:  :.:. : :* :*: : ...:.*    **  **********.**.

BsubAQ1FJ644630.1          GGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWT
BlichI5ABF61784            GGNYSVNWSNTGNFVVGKGWTKGSPSRTINYNAGVWAPNGNGYLALYGWT
P.catalpaeWP_091185626     GGNYSVNWSNTGNFVVGKGWNTGSASRVINYNAGVWAPSGNGYLTLYGWT
P.timonensis_              GGNYSVNWYNTGNFVVGKGWNVGSPNRTINYNAGVWAPSGNGYLTLYGWT
CM1                        GGNYSVTWGDTGNFVVGKGWEIGSPNRTIHYNAGVWEPSGNGYLTLYGWT
                           ******.* :**********  **. *.*:****** *.*****:*****

BsubAQ1FJ644630.1          RSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGDN
BlichI5ABF61784            RSPLIEYYVVDSWGTYRPTGTYKGTVYSDGGTYDIYTTKRYNAPSIEGQH
P.catalpaeWP_091185626     RNSLIEYYVVDSWGTYRPTGSYKGTVSSDGGTYDIYTTQRVNAPSIDG-T
P.timonensis_              RNSLIEYYVVDSWGTYRPTGTYKGTVTSDGGTYDIYTTMRYNAPSIDG-T
CM1                        RNQLIEYYVVDNWGTYRPTGTHRGTVVSDGGTYDIYTTMRYNAPSIDG-T
                           *. ********.********:::*** ********** * *****:*

BsubAQ1FJ644630.1          TTFTQYWSVRQSKRPTGSNAAITFSNHVNAWKSHGMNLGSNWAYQVLATE
BlichI5ABF61784            STFTQYWSVRRSKRPTGNNAKITFSNHVKAWKSHGMNLGSIWSYQVLATE
P.catalpaeWP_091185626     ATFTQYWSVRQSKRATGSNVAITFSNHVNAWKSKGMNLGSSWSYQVLATE
P.timonensis_              QTFQQFWSVRQSKRPTGSNVAITFSNHVNAWKSKGMNLGSSWSYQVLATE
CM1                        QTFQQFWSVRQSKRPTGNNVSITFSSHVNAWRNAGMNLGSSWSYQVLATE
                            ** *:****:***.**.*. ****.**:**:. ****** *:*******

BsubAQ1FJ644630.1          GYKSSGSSNVTVW…….71%
BlichI5ABF61784            GYQSSGSSNVTVW…….72%
P.catalpaeWP_091185626     GYQSSGSSNVTVW…..76%
P.timonensis_              GYQSSGSSNVTVW…..79%
CM1                        GYQSSGRSNVTVW….100%
                           **:*** ******
```

Fig 3 Alignment of CM1 xylanase GH11 compared to other xylanases. BsubAQ1fJ644630.1: xylanase GH11 from *Bacillus subtilis* AQ1; BlichI5ABF61784: xylanase GH11 from *Bacillus licheniformis* I5; P.catalpaeWP_091185626: xylanase from *Paenibacillus catalpae;* P.timonensis_: xylanase GH11 from *Paenibacillus timonensis.*

(Dundar 2015). The pJET1.2/blunt plasmid used in this study because this plasmid has a lethal restriction enzyme gene that is disrupted by ligation of a DNA insert into the cloning site. As a result, only bacterial cells with recombinant plasmids are able to form colonies. This positive selection drastically accelerates the process of colony screening (Thermo Scientific, 2015). After ligation of the PCR product into pJET1.2/blunt vector, there was only one colony grew on transformation plate. This show the low efficiency of transformation that can be caused by some factors like competency of cell, heat-shock protocol, ligation efficiency, and many more (Buwono *et al* 2018). The efficiency of the ligation process depends on the conditions of ligation reaction. Factors that influence the efficiency of ligation are buffer that used, reaction temperature, fragment end structure, and comparison of vector concentrations with target DNA. The isolation of this gene is the first step to study this gene. There are many things to do like study to know the suitable of the host and vector, the expression of the gene, and the characterization of enzyme of this gene.

The xylanase GH11 gene derived from *Bacillus halodurans* CM1 was successfully inserted the pJET plasmid and cloned in the host *Esherichia coli* DH5α. Based on the results of BLAST nucleotides there are similarities with genes of endo-1,4-beta-xylanhydrolase xyn11A) from *Bacillus halodurans* C-125 by 99%. Based on the resultof BLAST and the alignment of amino acid, CM1 xylanase GH11 had 71% and 72% identity with that from *B. subtilis* AQ1 and *B. licheniformis* I5, respectively (Helianti *et al.* 2010; Helianti *et al*. 2008).

## REFERENCES


Birnboim HC, Doly J. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7(6): 1513–1523. doi: 10.1093/nar/7.6.1513.

Brown TA. 2010. Gene cloning and DNA analysis: An introduction. Sixth Edition. Wiley - Blackwell: West Sussex.

Buwono ID, Iskandar, Agung UK, Subhan U. 2018. Buku Ajar Aplikasi Teknologi DNA Rekombinan Untuk Perakitan Konstruksi Vektor Ekspresi Ikan Lele Transgenik. Yogyakarta: Deepublish.

Dundar M. 2015. Current applications of biotechnology. Kayseri: Erciyes University.

Helianti I, Nurhayati N, Wahyuntari B. 2008. Cloning, sequencing, and expression of a β-1,4-endoxylanase gene from Indonesian *Bacillus licheniformis* strain I5 in *Escherichia coli*. World J Microbiol Biotechnol. 24(8):1273-1279. doi: 10.1007/s11274-007-9601-6.

Helianti I, Nurhayati N, Wahyuntari B, Ulfah M, Wahyuntari B, Setyahadi S. 2010. Constitutive high level expression of an endoxylanase gene from the newly isolated *Bacillus subtilis* AQ1 in *Escherichia coli*. J Biomed Biotechnol. 2010;2010:980567. doi: 10.1155/2010/980567.

Helianti I, Ulfah M, Noer S, Nurhayati N, Saefudin E. 2018. Cloning and expression of alkalophilic xylanase gene from an Indonesia Local *Bacillus halodurans* CM1 in *Escherichia coli* and its application on deinking process of waste paper. Malaysian J Microbiol. 14(7):655-662.

Hideto T, Kaoru N, Yoshihiro T, Go M, Rumie S, Noriaki M, Fumie F, Chie H, Yuka N, Naotake O,Satoru K, Koki H. 2000. Complete genome sequence of the alkaliphilic bacterium *Bacillus halodurans* and genomic sequence comparison with *Bacillus subtilis*. Nucleic Acids Res. 28(21):4317-4331. doi: 10.1093/nar/28.21.4317.

Inoue H, Nojima H, Okayama H. 1990. High efficiency transformation of *Escherichia coli* with plasmids. Gene. 96(1):23-28. doi: 10.1016/0378-1119(90)90336-p.

Jalal A, Rashid N, Rasool N, Akhtar M. 2009. Gene cloning and characterization of a xylanase from a newly isolated *Bacillus subtilis* strain R5. J Biosci Bioeng. 107(4):360-365. doi: 10.1016/j.jbiosc.2008.12.005.

Jeya M, Thiagarajan S, Lee JK, Gunasekaran P. 2009. Cloning and expression of GH11 xylanase gene from *Aspergillus fumigatus* MKU1 in *Pichia pastoris*. J Biosci Bioeng. 108(1):24-29. doi: 10.1016/j.jbiosc.2009.02.003.

Khan S, Ullah MW, Sidiqquie R, Nabi G, Manan S, Yousaf M, Hou H. 2016. Role of recombinant DNA technology to improve life. Int J Genomics. 2016:2405954. doi: 10.1155/2016/2405954.

Loenen WAM, Dryden DTF, Raleigh EA, Wilson GG, Murray NE. 2014. Highlights of the DNA cutters: A short history of the restriction enzymes. Nucleic Acids Res. 42(1):3 – 19. doi: 10.1093/nar/gkt990.

Moller S, McPherson M. 2000. PCR. New York: Taylor & Francis Group.

Safirah D, Helianti I, Kusumaningrum HP, Budiharjo A. 2016. Kloning dan sekuensing gen xilanase dengan produk gen berukuran 30 kDa dari *Bacillus halodurans* CM1 pada *Escherichia coli* DH5α. Bioma Berkala Ilmiah Biologi. 18(2):167-172.

Saito H, Miura KI. 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. Biochim Biophys Acta. 72:619-629.

Shahi N, Hasan A, Akhtar S, Siddiqui MH, Sayeed U, Khan MKA. 2016. Xylanase: A promising enzyme. J Chem Pharm Res. 8(3):334-339.

Singh R, Kumar M, Mittal A, Mehta PK. 2016. Microbial

enzymes: Industrial progress in 21st century. 3 Biotech. 6(2):174. doi: 10.1007/s13205-016-0485-8.

Sriyapai T, Somyoonsap P, Matsui K, Kawai F, Chainsiri K. 2011. Cloning of a thermostable xylanase from *Actinomadura sp*. S14 and its expression in *Escherichia coli* and *Pichia pastoris*. J Biosci Bioeng. 111(5)528-536. doi: 10.1016/j.jbiosc.2010.12.024.

Thermo Scientific. 2015. CloneJET PCR cloning kit: Product information. Carslbad: Life Technologies.

Ulfah M, Helianti I, Wahyuntari B, Nuhayati N. 2011. Characterization of a new thermoalkalophilic xylanase-producing bacterial strain isolated from Cimanggu hot spring, West Java, Indonesia. Microbiol Indones. 5(3):139-143. doi: 10.5454/mi.5.3.7.

Verma AS, Das S, Singh A. 2014. Laboratory manual for biotechnology. New Delhi: S. Chand & Company Pvt.