Jurnal Pertanian Tropik ISSN NO: 2356- 4725/p- ISSN : 2655-7576
Vol.7. No.1. April 2020 (11) 82- 93 DOI: https://doi.org/10.32734/jpt.v7i1.April.3826

Isolasi DNA Bakteri Pendegradasi Sulfat dari Tanah Masam dengan Metode Molekular

*DNA Isolation of The Cellulolytic Bacteria Degradation Strain (**Bacillus** sp) from Acid Soil by Molecular Method*

Eri Samah[1*], Milda Ernita[2]
[1.] Agrotechnology, Faculty of Agriculture Univ. Pembinaan Masyarakat Indonesia Medan
[2]. Agrotechnology, Faculty of Agriculture Univ. Tamansiswa Padang
**Corresponding author*: erisamah.2808@gmail.com

ABSTRACT

The purpose of this study was to obtain superior cellulolytic species and strains of bacteria to degrade organic waste into compost. The method used to isolate DNA by cultivating bacterial media Luria Bertani (LB), analysis of quality and quantity using DNA electrophoresis isolation technique visualized by electrophoresis technique followed by testing with Polymerase Chain Reaction (PCR) technique. Primary combination PCR amplification used 27F (5'-AGAGTTTGATCMTGGCTCAG-3 ') and 1525R (5'-AAGGAGGTGWTCC-ARCC-3'). Analysis of sequential data compared to gene sequences that have been deposited into public data bases in-silico uses the BLAST program which is conducted online NCBI (National Center of Biotechnology Information). The results obtained by visualization of electrophoresis from isolation of DNA 6 isolates of cellulolytic bacteria M = λ DNA 50 ng / µl; 1 = KM25, 2 = SR75, 3 = JM, 4 = U6,5 = G8,6 = K13. DNA concentrations obtained from 6 isolates were quite high ranging from 34.4 to 69.9 ng / µL. The results of the 6th PCR amplification of 6 isolates namely KM25, SR75, JM, G-8, KM13, AND U-6. In general, the six isolates of selected cellulolytic bacteria belong to the genus Bacillus. Phylogenetic analysis using Boostrap 1000 x. The six selected bacterial isolates analyzed separated and formed two main clusters: the first cluster which included Bacillus thuringensis and Bacillus cereus and the Bacillus Subtilis cluster. Conclusion The sequence of 6 isola is KM13 (Bacillus thuringensis), G8 (Bacillus cereus), Sr75 (Bacillus cereus), JM (Bacillus subtilis), KM25 (Bacillus subtilis), U6 (Bacillus subtilis).

Keywords: DNA Isolation, Molecular, Blast, Degradation Cellulolytic Bacteria

ABSTRAK

Tujuan penelitian ini untuk mendapatkan species dan strain bakteri selulolitik unggul untuk mendegradasi sampah organik menjadi kompos. Metode yang digunakanisolasi DNA dengan mengkultur bakteri media Luria Bertani (LB), analisis kualitas dan kuantitas menggunakan teknik elektrophoresis DNA hasil isolasi divisualisasi dengan teknik elektroforesis dilanjutkan uji dengan Teknik *Polymerase Chain Reaction* (PCR). Amplifikasi PCR kombinasi primer yang digunakan 27F (5'-AGAGTTTGATCMTGGCTCAG-3') dan 1525R (5'-AAGGAGGTGWTCC-ARCC-3'). Analisis data sekuens dibandingkan dengan sekuens-sekuens gen yang telah didepositkan ke dalam data base publik secara *in-silico* menggunakan program BLAST yang dilakukan secara *online* pada website NCBI (*National Centre of Biotechnology Information*). Hasil yang didapatvisualisasi elektroforesis hasil isolasi DNA 6 isolat bakteri selulolitik M=λ DNA 50 ng/µl; 1=KM25, 2=SR75, 3=JM, 4=U6, 5=G8, 6=K13. Konsentrasi DNA yang didapatdari 6 isolatcukuptinggiberkisar 34,4-69,9 ng/µL. Hasil amplifikasi PCR keenam 6 isolat yaitu KM25, SR75, JM, G-8, KM13, DAN U-6.Secara umum keenam isolat bakteri selulolitik terpilih tergolong kepada genus *Bacillus*. Analisis phylogenetik menggunkan Boostrap 1000 x. Keenam isolat bakteri yang terpilih yang dianalisis memisah dan membentuk dua cluster utama yakni: cluster pertama yang meliputi *Bacillus thuringensis* dan *Bacillus cereus* dan cluster *Bacillus Subtilis*. Kesimpulan sekuensingke 6 isola

tadalah KM13(*Bacillus thuringensis*), G8(*Bacillus cereus*), Sr75(*Bacillus cereus*), JM(*Bacillus subtilis*), KM25(*Bacillus subtilis*), U6 (*Bacillus subtilis*).

Kata Kunci: Isolasi DNA, Molekuler, Blast, Bakteri Sellulolitik Degradasi

INTRODUCTION

Cellulolytic degradation is a bacterium that is found in many soils that functions as a decompuser of coarse-fiber organic waste. Cellulolytic degradation bacteria can degrade finely fibrous to organic waste into compost because these bacteria contain cellulase enzymes. For more details, the working order of these enzymes can be seen in Figure 1.

Cellulolytic bacteria degradation from ultisol acid soil of Andalas University in Padang based on the test of the clear zone index, which is the ratio between the diameter of the bacterial clear zone (cm) and the diameter of the bacterial colony (cm), has been found by Samah (2019), namely 6 superior isolates KM25, SR75 JM, KM13, G8, and U6 of 24 isolates selected based on the size of the clear zone index respectively 3.12; 3.04; 2.03; 2.04; 2.00; and 2.04. This research is a continuation of the dissertation research which is continued by molecular DNA analysis.

DNA isolation is an initial step that must be understood before carrying out molecular analysis. The basic principle of DNA isolation is the attempt to free genetic material from the cell wall and the binding of histone proteins which are mainly located in the cell nucleus by making minimal mechanical or physical damage to the genetic material. DNA obtained from the results of isolation is not pure DNA but there is a mixture of RNA and other types of proteins with different molecular weights with DNA, hence to do the separation of DNA molecules is done by electrophoresis techniques. Electrophoresis technique is one of the molecular separation techniques using electric current that utilizes the principle of large/molecular weight differences (Jamsari, 2007).

DNA produced from electrophoresis can then be sequenced, but beforehand PCR amplification is done first (Jamsari, 2007). Based on the study of Walida et al ,. (2019) has carried out the isolation and identification of IAA-producing bacteria and phosphate solvents from the rhizosphere of the Palm Oil Plant and on the isolation and antagonism of local microorganisms (mol) bamboo shoots on the fungus Fusarium sp. The PCR reaction mimics the DNA replication or replication reaction that occurs in living things. Simply put, PCR is a reaction doubling certain regions of printed DNA with the help of the enzyme DNA polymerase (Trisnawati A, 2013).

Determination of RNA sequences is usually done by sequencing the printed DNA. Today, almost all DNA sequencing efforts are carried out using the chain termination method developed by Frederick Sanger and his colleagues. The technique involves termination or stopping the reaction of DNA synthesis by in vitro that is specific to a particular sequence using a modified nucleotide substrate (Suryanto, 2003 cit Andesti, 2010).

The effectiveness of gliocladium virens in controlling Fusarium oxysporum F. sp. Capsici disease in chilli plants (Afriani, A, et al., 2019). Sequence analysis is a technique that is considered best for looking at the biodiversity of a group of organisms. This technique developed after people invented the DNA sequencer engine. Identifying species diversity can be done through analysis of 16S-rRNA gene sequences for prokaryotic organisms or 18S-rRNA for eukaryotic organisms. According to Tallei et al. (2016), the less genetic distance between two organisms, the closer the kinship between the two.

 ISSN NO: 2356- 4725/p- ISSN : 2655-7576
Vol.7. No.1. April 2020 (11) 82- 93 DOI: https://doi.org/10.32734/jpt.v7i1.April.3826

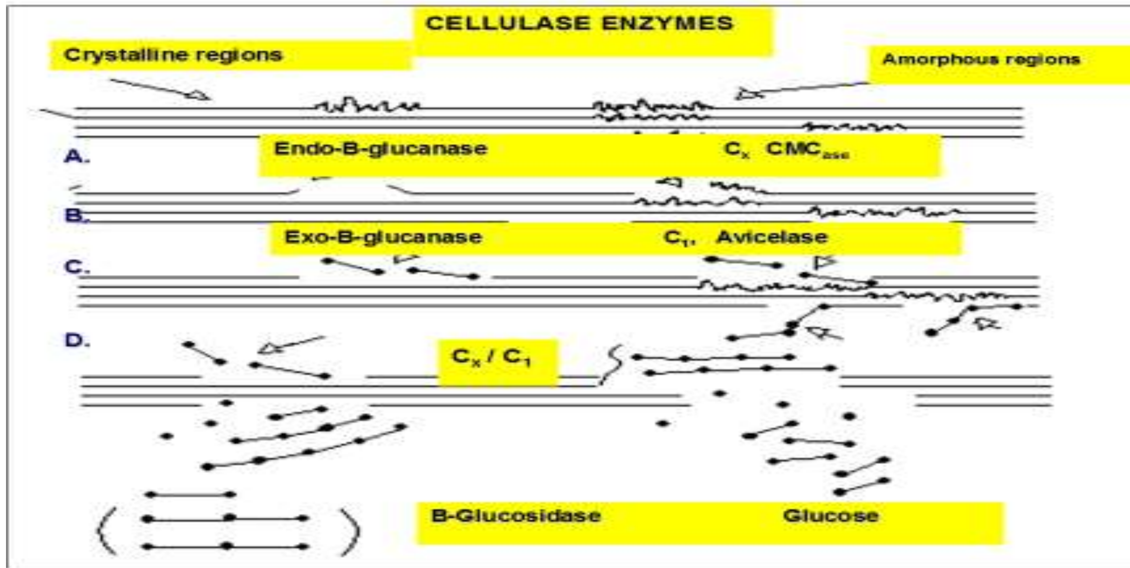

Figure 1. Cellulolysis circuit schematic (Reese et al., 1972)

Comparison of rRNA sequences is a good tool for reducing phylogeny relations. Certain enzyme-producing genes, for example, can also be compared based on sequences. At present databases for many 16S-rRNA and 18S-rRNA genes are available and stored for example in Gene-Bank, and can be accessed for example through http://www.ebi.ac.uk. Likewise for many important enzyme-producing genes and several other sequences (Jamsari, 2007).

Analysis of genetic variation can be done morphologically, physiologically, biochemically and molecularly. Molecular analysis can be based on DNA banding patterns or nucleotide sequences. Analysis of DNA bands or by fingerprinting, analysis of DNA sequences produces polymorphisms based on the sequence of nucleic acids. Analysis carried out by comparing on line can also be done using internet services from gene banks on the NCBI (National Center of Biotecnology Information) BLAST program on the web site http://www.ncbi.nlm.nih.gov/BLAST (Jamsari, 2007).

MATERIALS AND METHODS

Cellulolytic bacterial identification is carried out by descriptive method, namely by taking data at the Agricultural Biotechnology Laboratory of UNAND, with the following stages:

a. DNA Isolation

DNA isolation is done by culturing bacterial colonies from the results of bacterial selection in the testing of extra cellular extracts. The working procedure of bacterial DNA isolation is based on the method of Jeff Newman (Hazar et al., 2015). The initial activity of bacterial DNA isolation is culturing bacterial colonies which are done by taking a single bacterial colony from the dissertation pre-study (Samah, 2019) and transferred into LB (Luria Bertani) media using a toothpick. Furthermore, the bacterial culture was then shaken at a speed of 160 rpm for ± 24 hours (overnight).

The following work procedures are carried out as follows. A total of 10 ml of liquid culture of isolates that had been bred for ± 24 hours were put into 2 ml eppendorf tubes, centrifuged for 30 minutes at 14,000 rpm to get pellets. The pellet was resuspended with 1 x TE (Tris-EDTA) of 500 µL. Then added 50 µL 10% SDS and 5 µL proteinase K (10 mg/mL). The mixture is flipped to complete and then incubated for 1 hour at 37°C. After that it is added with a mixture of Phenol: Chloroform (PC) (24:24) as much as 1 x volume and centrifuged for 3 minutes at a speed of 14,000 rpm. Then the supernatant

ISSN NO: 2356- 4725/p- ISSN : 2655-7576
Vol.7. No.1. April 2020 (11) 82- 93
DOI: https://doi.org/10.32734/jpt.v7i1.April.3826

(top) is taken and transferred into another 2 ml eppendorf tube.

Then add once more PC mixture of the same volume and centrifuged for 5 minutes at 14,000 rpm. The supernatant is removed and transferred back into a new sterile 1.5 ml eppendorf tube. Then added 1/10 volume of 3 M sodium acetate and also added 0.6 volume of cold isopropanol and stirred by flipping the tube until the DNA is precipitation. Furthermore, centrifuged for 1 minute at a speed of 14,000 rpm. The DNA pellets were washed with 70 ml of cool ethanol as much as 1 ml for 30 seconds and dried using a heater block at 55^0C for 5 minutes. After that the DNA pellet is suspended with 1 x TE buffer of 100 µl, then the quality and quantity are analyzed using electrophoresis techniques.

b. DNA Quality and Quantity Analysis

DNA solutions obtained from DNA isolation were prepared for quality and quantity analysis using electrophoresis techniques. Agarose gel with a concentration of 1% was used as a matrix for this analysis. Melting agarose gel is added to the compound ethidium bromide (5 mg/ml) and allowed to harden in the fume hood. Other gel well solutions are also included DNA-λ with known concentrations (25 ng/µl) and used as a reference for determining concentrations.

Electrophoresis was run at a voltage of 100 V for 1 hour using a 0.5x TBE buffer (Tris-Boric-EDTA). After completion, the gel was exposed to UV light under a documentation device (Gel-Doc) (Biometra-German). Visualization data is stored in the form of digital data and is used for the analysis of the quality and quantity of DNA. The obtained DNA is then diluted to a concentration of 5 ng/µl which will be used as a working solution. The remaining DNA solution is used as a reserve stock solution in the next analysis.

c. 16S rRNA Gene Amplification

The isolated DNA was amplified using a primer combination designed from the 16S rRNA gene sequence. The primary combinations used are 27F (5'-AGAGTTTGATCMTGGCTCAG-3 ') and 1525R (5'-AAGGAGGTGWTCC-ARCC-3 ') (Wowrik et al., 2005). This gene region is widely used in phylogenetics, classification, and identification for bacteria because of their ubiquitous nature with identical functions in all bacteria. Amplification with this primer is estimated to produce a PCR product of around 1500 bp.

The PCR reaction was carried out at a total volume of 25 µl consisting of 2 µl DNA templates, 2 µl combinations of 27F and 1525R primers (5 pmol/µl), 21 ul ddH2O PCR in RTG - PCR Bead, respectively. The PCR conditions used were: denaturation (94^0C) for 1 minute, annealing at 57^0C for 1 minute, and an extension for 1 minute at 72^0C. An additional extension was carried out at 72ºC for 5 minutes. The amplification results are stored at 4ºC before use. To control the success of the PCR product amplification reaction as much as 5 µl then electrophoresis was applied to agarose gel with a concentration of 1% in a 0.5x TBE buffer at 100 Volt voltage (Maniatis et al., 1989).

Visualization using UV-transilluminator after staining ethidium bromide and documented into digital data in JPEG format. The remaining 20 µl PCR products were stored at -20^0C for sequence analysis purposes.

d. **16S rRNA Sequence Analysis**

PCR products are sent to the sequencing service company in two directions using primers 27F and 1525R. For sequencing purposes, 20 µl PCR products were used with concentrations of 25 mg µl, while the primers used were 27F and 1525R with concentrations of 5 pmol/µl with a volume of 10 µl. The 16S rRNA gene sequencing process obtained from PCR amplification was carried out at Singapore's 1st Base company.

e. **Data Analysis**

Analysis of sequence data is compared with gene sequences that have been deposited into public databases by in-silico using the BLAST program conducted online on the

NCBI (National Center of Biotechnology Information) website: http://www.ncbi.nlm.nih.gov/BLAST

RESULT AND DISSCUTION

Isolation and Identification of Selected Cellulolytic Bacteria by Molecularly

Cellulolytic bacteria that have been selected in stage II and have been identified both morphologically, gram staining and biochemistry are continued to be identified molecularly. There are 6 bacterial isolates that are identified molecularly based on the clear zone index that produces ≥ 2.0. The six isolates were KM25; SR75; JM; KM13; G-8 and U-6.

a. DNA Isolation

DNA isolation was carried out using the Jeff Newman method. The working procedure for DNA isolation can be seen in the materials and methods section. The isolated DNA was visualized by electrophoresis using 1% agarose in a TBE (Tris Boric-EDTA) buffer with ethidium bromide staining. DNA electrophoresis was carried out at a voltage of 100 volts for 30 minutes and DNA fragments were observed using a UV transilluminator. As a comparison, λ DNA was used with a concentration of 50 ng/ul. DNA concentration is determined by comparing the level of intensity of isolated DNA fragments. The results of DNA isolation are presented in (Figure 2).

Observing at the appearance of DNA electrophoresis resulting from the isolation activities of the six selected cellulolytic bacterial isolates, it appears that all isolates were successfully isolated. This is indicated by the emergence of a main fragment that is firmly aligned with DNA λ. Besides, the lack of smear fragments indicates that the isolation protocol chosen is effective for use. Fragment intensity although different, but in general, they have a high enough concentration when compared with DNA λ that is used as a reference.

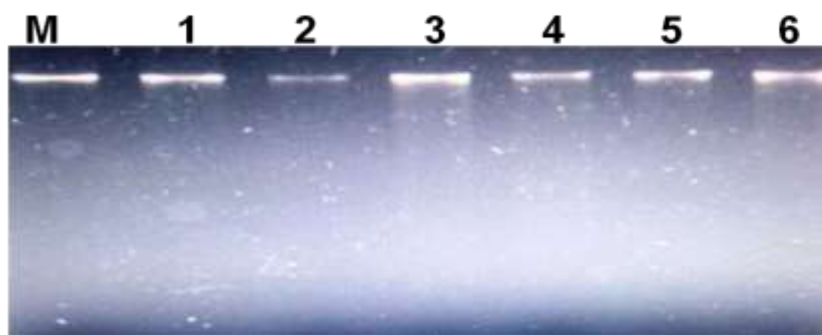

Figure 2. Visualization of electrophoresis from DNA isolation of 6 cellulolytic bacterial isolates M = λ DNA 50 ng/µl; 1 = KM25, 2 = SR75, 3 = JM, 4 = U6, 5 = G8, 6 = K13

Table 1. The concentration of 6 DNA isolates of cellulolytic bacteria using NanoDrop

Isolate Code	DNA Concentration (ng/µl)	A260	A280	A260/ A280	DNA Criteria
JM	49,8	0,995	0,524	1,9	Murni
KM25	69,9	1,398	0,754	1,85	Murni
G8	50,5	1,01	0,543	1,86	Murni
KM13	63,6	1,271	0,634	2,01	Murni
SR75	60,7	1,213	0,576	2,11	Murni
U6	34,4	0,688	0,340	2,02	Murni

Observing at the appearance of DNA electrophoresis resulting from the isolation activities of the six selected cellulolytic bacterial isolates, it appears that all the isolates were successfully isolated. This is indicated by the emergence of the main fragment that is firmly aligned with DNA λ. Besides, the lack of smear fragments indicates that the isolation protocol chosen is effective for use. Fragment intensity although different, but in general, they have a high enough concentration when compared with DNA λ that is used as a reference.

b. Measurement of DNA Concentration Using NanoDrop

To ensure accurate isolation of DNA concentrations, measurements were taken using ultraviolet ray spectrophotometric platforms. The principle used in this case is, the amount of radiation absorbed by DNA is directly proportional to the amount of DNA in the sample. The concentration is declared high if more than 10mg/ml. Nitrogen bases absorb UV light, the higher the concentration of DNA solution, the more UV light is absorbed.

The concentration of double-stranded pure DNA with A260 of 1.0 was 50 mg/ml. Thus, the above rules can be used to determine the formula of DNA concentration in a DNA solution and also related to DNA quality. The absorption ratio at 260 nm and 280 nm was used to assess the purity of DNA and RNA. DNA is declared pure if it has an OD260/OD280 ratio value ranging from 1.8-2.0. It is classified as high, ranging from 34.4 to 69.9 ng/µL.

Table 4 showed the concentration of DNA obtained from the results of isolation activities. The concentration was high and very sufficient for the needs of the next stage of analysis using the in-vitro amplification technique (PCR). The quality of DNA obtained from the three isolates of JM, KM25, and G8 is classified as pure, which is shown by the ratio of OD260/OD280 ranging from 1.8 to 2.0, while for isolates KM13, SR75 and U6, the ratio of OD260/OD280 is slightly greater than the maximum value 2.0, which were 2.01, 2.11, and 2.02, respectively. If the ratio value is greater than 2.0 then the quality of DNA is considered to be no longer pure, possibly contaminated with proteins or other compounds.

c. Polymerase Chain Reaction (PCR) Using Primers 27F and 1525R

The Polymerase Chain Reaction (PCR) technique is a method for enzymatic amplification of DNA fragments. Amplification activity was carried out using a combination of primers 27F and 1525R which were expected to be able to amplify the 16S-rRNA gene sequence area. The use of these primary combinations is expected to produce PCR product fragments of about 1500 bp. Complete procedures and components for the amplification reaction can be seen in the materials and methods. The results of visualization were carried out using electrophoresis techniques on agarose gels, with a voltage of 100 volts and smoothed for 30 minutes. Figure 3 showed that from the 6 isolates analyzed by PCR technique, 4 isolates were able to produce fragment products according to expectations with very good intensity. Two isolates namely KM13 and JM produced small concentrations but after sequencing it turned out that the 2 isolates were able to produce reasonably good sequence data (Figure 4).

d. Analysis of Selected Cellulolytic Bacteria 16S rRNA gene sequences

To determine the isolate of cellulolytic bacterial isolates that have been isolated and determine their variation and kinship, sequencing analysis was carried out on the results of the 6 isolates PCR amplification results, namely KM25, SR75, JM, G-8, KM13, and U-6.

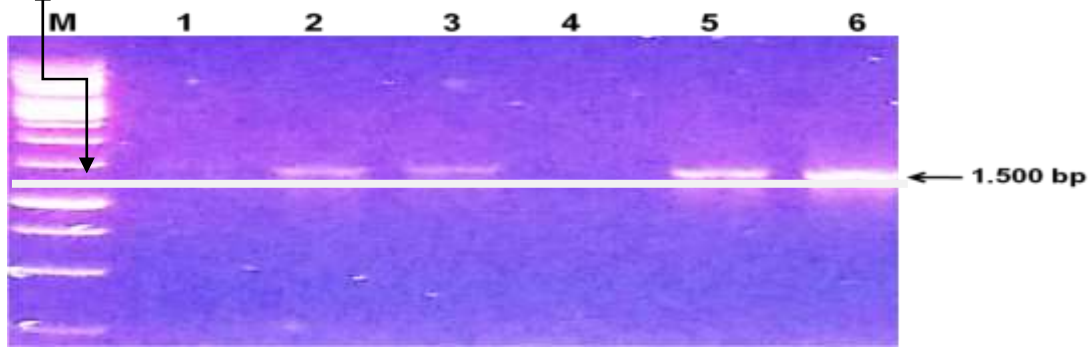

Figure 3. Visualization of 16S rRNA gene PCR products from 6 isolates of cellulolytic bacteria using a combination of primers 27F and 1525R. M = 1kb ladder, 1 = KM13, 2 = G8, 3 = SR75, 4 = JM, 5 = KM25, U6

Figure 4. Electrophoregram Sequence of 6 cellulolytic bacterial isolates selected after editing

ISSN NO: 2356- 4725/p- ISSN : 2655-7576
Vol.7. No.1. April 2020 (11) 82- 93 DOI: https://doi.org/10.32734/jpt.v7i1.April.3826

In Figure 4, analysis with sequencing techniques is considered more accurate in both prokaryotic and eukaryotic organisms. Compared with PCR-RAPD-based molecular analysis, the information obtained from sequencing data is more consistent (Hidayat et al., 2004). Sequencing is done bi-direction (two sides) using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3 ') and 1525R (5'-AAGGAGGTG-WTCCARCC-3 ').

Sequence data obtained from the six 6 samples are then edited using the assistance of a software package and manually controlled. From the 6 samples analyzed there is 1 sample that cannot be edited simultaneously between forward and reversion hence each must be edited that is the SR75 sample. The testing results of the DNA purity level of SR75 isolates also included less pure in which the A260/280 ratio was 2.11, this is due to lack of clean DNA contained therein. If the ratio value is greater than 2.0 then the quality of DNA is considered to be no longer pure, possibly contaminated with proteins or other compounds (Samah, 2019 - Table 4). Besides these isolates, the five other isolates produced good sequence data (Figure 4).

e. BLAST Analysis

The BLAST analysis is carried out to compare the sequence data obtained with DNA sequences of various organisms from all over the world, especially bacteria deposited on the database (gene bank) of public sequences. For this purpose, the BLAST analysis is carried out online on the NCBI (National Center of Biotechnology Information) website. The results obtained from 6 collections of cellulolytic bacterial isolates based on BLAST analysis can be seen in Table 1.

BLAST identification results in Table 2 generally showed that the six selected cellulolytic bacterial isolates belong to the genus Bacillus. Huda (2010) got different results, where from 6 isolates of cellulolytic bacteria studied, 5 isolates belonged to the Bacillus group while one isolate was classified as Uncultured bacterium. The results of the BLAST analysis which included the six isolates selected as Bacillus sp. are in line with the morphological observations. Yusuf (2000) reported that the genus Bacillus if cultured in Nutrient Agar (NA) medium would be shiny white to slightly creamy, round, oval to irregular in shape, the surface of the jagged flat colonies and scattered on the surface of the medium and gram tests found that the bacteria were gram-positive.

In addition, bacteria that approach the genus Bacillus usually have morphological characteristics as follows: the color of the colony is milky white or slightly creamy, the shape of the colony is round, with a wrinkled edge. The shape of cells is like a stem and straight, measuring of 0.5 - 2.5 x 1.2 - 10 µm, and often arranged in the form of a pair or two chains, with round or square edges which can be seen in (Figure 5). Staining of gram-positive cells, Motyl, Catalase and Oxidation are positive. Methyl red is negative, optimum at temperatures of 30-7ºC and growing well on NaCl 1-3. (Feliatra et al., 2004).

f. Genetic kinship of cellulolytic bacteria based on 16S-rRNA encoding gene sequences

The genetic kinship analysis of cellulolytic bacteria was carried out on six sequential isolate data from a sample collection of selected cellulolytic bacterial isolates. The sequence data used are arranged in FASTA format in notepad. Then the data is transferred to the CLUSTALW version 1.83 data entry format, which is done online through the website: http://www.genebee.msu.su/clustal/-advanced.html.

Figure 5, showed phylogenetic analysis using Boostrap 1000, means that the repetition is up to 1000 times. The six selected bacterial isolates analyzed separated and formed two main clusters: the first cluster which included Bacillus thuringensis and Bacillus cereus and also the Bacillus Subtilis cluster. SR75 isolates were identified as Bacillus sp species with 19% node and if pulled back Bacillus cereus species MF code 187565 node 42%.

Jurnal Pertanian Tropik ISSN NO: 2356- 4725/p- ISSN : 2655-7576
Vol.7. No.1. April 2020 (11) 82- 93 DOI: https://doi.org/10.32734/jpt.v7i1.April.3826

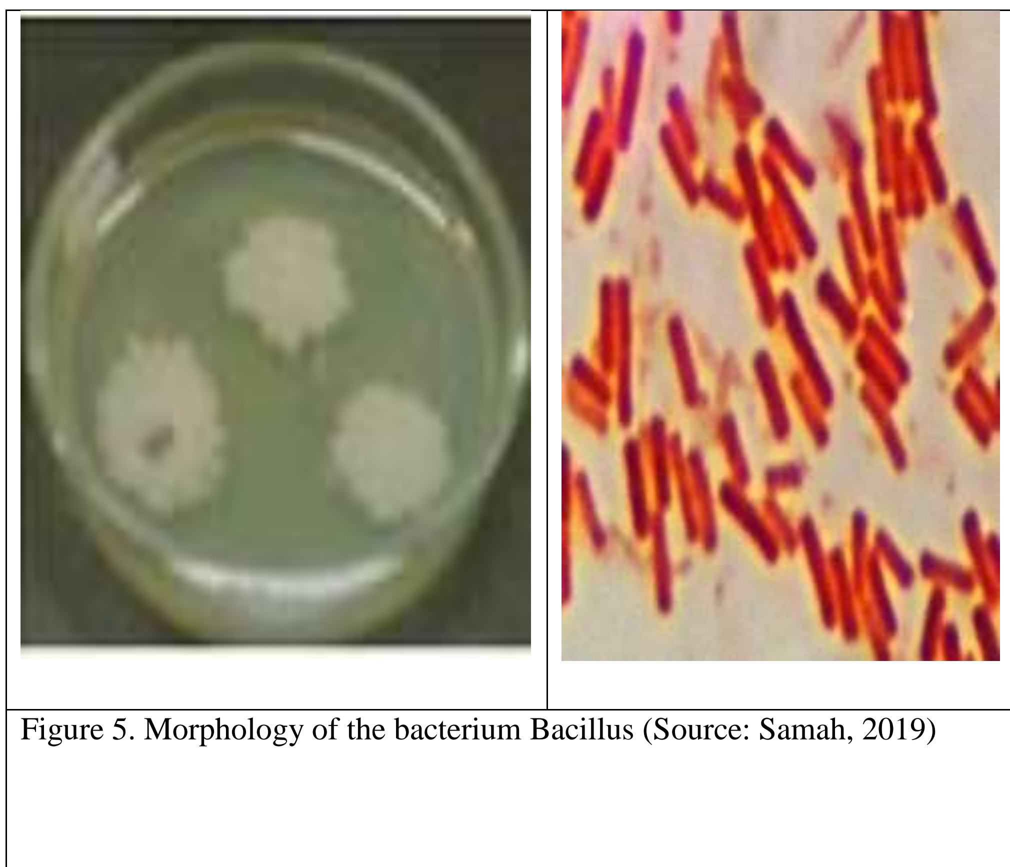

Figure 5. Morphology of the bacterium Bacillus (Source: Samah, 2019)

Table 4. Results of BLAST analysis of six selected cellulolytic degradation bacterial isolates.

No	Isolate Code	Possible species	Sequence Length (bp)	Access Code	*Query covera Ge (%)*	*Max ident (%)*
1	Km13	*Bacillus thuringensis*	1429	JF512478	97	97
2	G-8	*Bacillus cereus*	1328	MF526969	99	99
3	Sr75	*Bacillus cereus*	383	MF187565	99	99
4	JM	*Bacillus subtilis*	1292	KF135457	99	99
5	Km25	*Bacillus subtilis*	1347	EF101707	95	99
6	U-6	*Bacillus subtilis*	1394	GU227615	99	99

Note, Access code = the compared databank access code. Query coverage = percentage of similarity of the sample with the database. Max ident = information level of similarity between the sample sequence and the access sequence.

Jurnal Pertanian Tropik ISSN NO: 2356- 4725/p- ISSN : 2655-7576
Vol.7. No.1. April 2020 (11) 82- 93 DOI: https://doi.org/10.32734/jpt.v7i1.April.3826

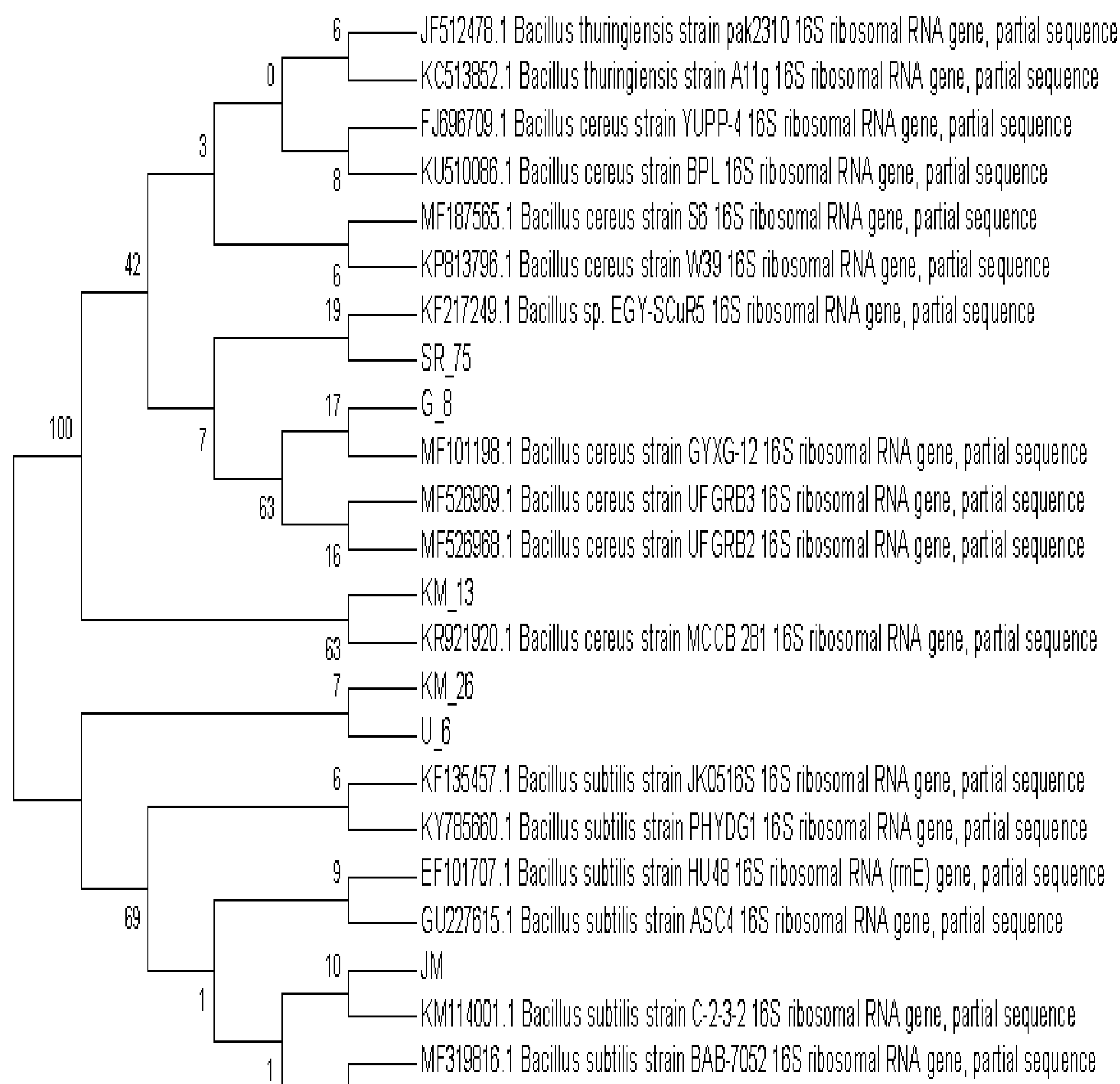

Figure 6. Phylogenetic 6 isolates of cellulolytic bacteria (SR 75, G-8, KM13, KM25, U-6, and JM).

G-8 isolates including Bacillus cereus species with code 101198 node 17%. KM13 isolates also included Bacillus cereus species with code KR921920 node 63%. KM25 isolates have a close relationship with U-6 identified as species of Bacillus subtilis strain KF 135457 node 69%. JM isolates including Bacillus subtilis species strain KM 114001 node 10%.

The higher the node level or the repetition rate (%), the higher the level of accuracy.

CONCLUSION

Based on this research, it can be concluded that from 6 isolates of cellulolytic bacteria KM25, SR75, JM, U6, G8, and K13, the DNA was successfully isolated at a concentration of 50 ng/µl, and when measured using NanoDrop, the six isolates stated as pure because it has an OD260-/OD280 ratio value ranging from 1.8-2.0. After the amplification was done using Polymerase Chain Reaction (PCR) with Primary 27F and 1525R fragments 1500 bp and continued with sequencing, the six isolates were obtained. The 6 sequences of isolates were KM13 (Bacillus thuringensis), G8 (Bacillus cereus),

Jurnal Pertanian Tropik ISSN NO: 2356- 4725/p- ISSN : 2655-7576
Vol.7. No.1. April 2020 (11) 82- 93 DOI: https://doi.org/10.32734/jpt.v7i1.April.3826

Sr75 (Bacillus cereus), JM (Bacillus subtilis), KM25 (Bacillus subtilis), U6 (Bacillus subtilis).

Acknowledgement

The writers wanted to say their gratitude to the Ministry of research and technology of higher education which has helped the fund of this research in the form of a decentralized grant schema doctoral dissertation contract number 055/KI.I/AT.I/2015, March 10, 2015.

REFERENCES

Afriani, A., Heviyanti, M. and syawal Harahap, F., 2019. Effectiveness of gliocladium virens in controlling Fusarium oxysporum F. sp. capsici disease on chilli plant. *Jurnal Pertanian Tropik (Indonesian Tropical Agriculture Journal) accredited by KEMENRISTEK DIKTI No: 21/E/KPT/2018*, *6*(3, Dec), pp.403-411.

Ariwibawa, Nugraha 2004. Erodibilitas Tanah Di Kecamatan Jogorogo Kabupaten Ngawi Propinsi Jawa Timur. Skripsi. Surakarta : Fakultas Geografi UMS

Feliatra. 2004. Isolasi dan Identifikasi Bakteri Probiotik dari Ikan Kerapu Macan (Ephinephelus fuscogatus) dalam Upaya Efisiensi Pakan Ikan. Jurnal Natur Indonesia. 6 (2): 74-81.

Hazar, M., Salim, M., dan Mardiah, E. 2015. Keberadaan *Escherichia Coli* Resistan Antibiotik Pada Ikan Balang (*Pristolepis Fasciata*) Di Sungai Batang Arau Jurusan Kimia, Fakultas MIPA, Universitas Andalas Limau Manis, Padang.

Hidayat P, Aidawati N, Hidayat SH, Sartiami D. 2008. Tanaman indikator dan teknik RAPDPCR untuk penentuan biotipe Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae). Jurnal Hama dan Penyakit Tumbuhan Tropika 8:1–7.

Huda, B.I. 2010. Koleksi dan Identifikasi Bakteri Selulolitik dari Kawah Gunung Merapi Berdasarkan Analisis Sekuens Gen Pengkode 16S-rRNA. Skripsi Fakultas Pertanian Unand. 16 Pages

Jamsari. 2007. Bioteknologi Pemula. Prinsip Dasar dan Aplikasi Analisis Molekuler. Unri Press. Page 46-48.

Reese, E.T., R.G.H. Siu and H. S. Levinson. 1972. *in* :W. M. Fogarty (*ed*). 1983 Microbial Enzymes and Biotechnology. Ap plied Scence Publ., London.soil as a potential candidate of organic waste degradation

Samah E. dan Misdawati. 2019. Isolation of Cellulose Degradation Bacteria (CDB) from acid soil. Jurnal Tropika USU. Vol.6.No3. ISSN NO: 2356-4725/p- ISSN : 2655-757. https://talenta.usu.ac.id/jpt.

Sambrook, J. ; Fritsch, E. F. ; Maniatis, T. 1989. Molecular cloning: a laboratory manual. Tex Book No.Ed. 2 pp.xxxviii + 1546 pp

Suryanto, Dwi. 2009. Prospek Keanekaragaman Hayati.

Tallei, T.E., Rembet, R.E., Pelealu, J.J., Kolondam, B.J. 2016. Sequence Variation and Phylogenetic Analysis of Sansevieria trifasciata (Asparagaceae). Bioscience Research 13(1): 01-07.

Trisnawati E, Andesti D, Saleh A. 2013 Pembuatan Kitosan Dari Limbah Cangkang Kepiting Sebagai Bahan Pengawet Buah Duku Dengan Variasi Lama Pengawetan", Jurnal Teknik Kimia No. 2, Vol. 19,

Walida, H., Harahap, F.S., Hasibuan, M. and Yanti, F.F., 2019. Isolasi dan Identifikasi Bakteri Penghasil IAA dan Pelarut Fosfat dari Rhizosfer Tanaman Kelapa Sawit. *BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan)*, *6*(1), pp.1-7.

Weisberg, S.P. McCann D, Desai M.Rosenbaum M, Leibel RL, Ferrante AW (2003). Obesitas is associatedwith macrophage accumulation in adipose tissue.*J Clin. Invest 112*, 1796–1808..\

Wowrik B, Kerkhof L, Zylstra GJ, Kukor JJ. 2005. Identification of unique type II Polyketid synthase gene is soil. Appl Environ Microbiol. 71(5):2232-2238. doi: 10.1128/ AEM.71.5.2232-2238.2005.

Yusuf, S. 2000. Bakteri serasah yang terdapat di hutan gambut ditinjau dari segi daerah tertutup dan terbuka. Skripsi Sarjana Biologi. FMIPA. Universitas Andalas. Padang