ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Identification and Phylogenetic Diversity Based on 16S rRNA Gene Sequence Analysis of Thermophilic Bacteria from Rimbo Panti Hot Spring.

Armaini[a*], Abdi Dharma[a], Sumaryati Syukur[a], Jamsari[b], and Tjong Hon Djon[c].

[a]Biochemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, 25163, Indonesia.
[b]Faculty of Agriculture, Andalas University, Padang25163 Indonesia.
[c]Department of Biology Faculty of Mathematics and Natural Sciences, Andalas, University, Padang25163 Indonesia.

ABSTRACT

The Thermophilic bacteria were isolated from hot springs Rimbo Panti West Sumatera Indonesia. Screening is done by using a selection medium for thermophilic bacteria and to examine the potential of cellulases used medium containing CMC and stained with Congo red were obtained 14 isolates which marked the formation of clear zones. Identification of bacterial isolates based on, the Manual of Determinative Bacteriology Bergey's. Morphological observations of colonies were obtained cells rod shape and Gram staining of the 14 isolates was Gram positive, has endospores. Biochemical tests (catalase, VP, hydrolysis of starch, citric and growing at 6.5% NaCl). To determine the species of these isolates to be identified using 16S rRNA, using primer combination, 27F and 1525R. PCR products were obtained 1500 bp fragment, sequencing of product PCR and BLAST The results of the identification can be concluded isolates of thermophilic bacteria is a Bacillus licheniformis, Bacillus cereus, Bacillus subtilis and Bacillus sp.

Keywords: Thermophilic Bacteria, hot spring, Identification, sequencing.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Thermophilic microorganisms can be defined as organisms that live at high temperatures and not only survive but also to grow and thrive in boiling water [1-4] Thermophilic microorganisms can thrive in habitats where the temperature reaches 140 ° C (284 ° F), or the extreme-enzyme condition, in which the enzyme is directed to work at very high temperatures, amino acids extreme-enzyme has a special ability to maintain the three-dimensional structure of twisting and folding in high temperatures, where other enzymes are no longer working [4]. Cellular component of thermophilic microorganisms such as proteins are generally more stable than conventional microorganisms (mesophilic). Stable to heat is the specific character of these microorganisms [5]. Thermophilic bacteria can live at high temperatures, this is due to bacteria thermophilic have characteristics, which do not contain peptidoglycan in the plasma membrane but is only composed of lipids that have an important role in regulating the activity of the plasma membrane. Beside that also the plasma membrane of bacteria thermophilic contain more, saturated long-chain fatty acids that will increase the melting point of the plasma membrane so that it has a high stability against high temperatures due to the high melting point has a level] of fluidity plasma membrane at high temperatures required in the function [6].

METHODS

Sampling and Screening of thermophilic bacteria

Water samples taken from Rimbo Panti hot springs, West Sumatera, Indonesia. Screening is done by using a selection medium for thermophilic bacteria [7]. Water samples (5 ml) poured into Petri and add selection medium (10 ml), incubation at 70 º C for 2 days and 50 º C for 3 days. Each colony was separated and grown on selection medium to obtain single colonies on each Petri's at the same incubation temperature. Cellulase activity observed with the formation of clear zone using a medium for the formation of clear zone containing CMC and with Congo red dye. Cultivate every single isolates into each dish and incubation at 50 ° C for 2 days

Identification of thermophilic bacteria

That is based on the Manual of Determinative Bacteriology Bergey]8] is based on morphological observations of colonies of thermophilic bacteria is done by two ways, namely the observed macroscopic and microscopically identification, endo spores and Gram staining test by Christian Gram

Isolation of DNA from Thermophylic Bacteria

Isolation of DNA from thermophylic bacteria uses the same method performed by Jeff Newman (http://lyco.lycoming.edu/-newman).The quality and quantity of DNA analyzed by electrophoresis techniques.

Amplification using primer of 16S rRNA gene by PCR

Result of isolation DNA from thermophylic bacteria was amplification using primer combinations in the design of 16S rRNA gene sequences. The combination of primers used were 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGT GWTCCARCC-3'). PCR amplification products expected to generate approximately 1500 bp.. Amplification performed a total of 30 cycles with the PCR machine, operational condition : denature at 94 º C for 1 min, annealing (57 º C) for 1 minute, extension (72 º C) for 1 min, and extension (72 º C) added for 5 minutes, the amplification is stored in a temperature of 4 º C before use. To control the success of the reaction amplification, as much as 5 µl PCR products analyzed on agar rose (3 %) of electrophoresis

RESULTS AND DISCUSSION

Screening and Phenotypic identification of Thermophilic Bacteria

Screening thermophilic bacteria Base on potential of cellulose activity was found 14 isolate, which marked the formation of clear zones (Fig. 1). The identification of 14 bacterial isolates belonging to the genus Bacillus, gram-positive bacteria, has endospores (Fig. 1), aerobic / facultative aerobic and rod-shaped.

ISSN: 0975-8585

Endospores elliptical and circular (ring), the position of endospores in the end and in the middle. From the results of biochemical tests [7] obtained 3 Bacillus species gave a positive reaction to catalase, hydrolysis pati.Voges Proskauer (VP), citrate, grew 7% NaCl and grown at 55 ° C, these results are also compared to some other researchers (Table 1), it was concluded that this bacteria, Bacillus licheniformis, Bacillus cereus, Bacillus subtilis and Bacillus sp. Based on the results of the study [9] thermophilic bacteria form endospores and are aerobic.

Table 1: Results of biochemical tests, Gram stain and spore formation of 14 isolates of thermophilic bacteria

	A	B	C	D	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gram Staining	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Position Spores	C	C	C	C/T	T	T	C	C	T	C	T	T	T	T	T	T	T	T
Spore Shape	E	E	E	E/L	E	E	L	L	E	L	E	E	L	L	L	L	L	L
Cell Shape	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Grow at																		
45ºC	+	+	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50ºC	+	+	#	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
55ºC	+	+	#	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grow at NaCl 7%	+	+	#	+	+	+	-	-	+	-	+	+	-	-	-	-	-	-
Aerob/facultative	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid Formation																		
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	-	-
Manose	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	+	+
Citrate Test	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	-	-	+
Urease Test	-	-	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#
Indole	-	-	#	#	-	-	+	-	-	+	-	-	+	-	+	-	+	-
VP (Voges Proskauver)	-	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	-
Reducing Nitrate	+	+	+	+	+	+	+	+										
Strach Hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Produce oxidase	-	-	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#
Produce Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

A = Vaseekaran (2010) [14] B = Barrow and Feltham, (1993) C = Al-Janabi (2006) D = Bergeys (1974) [7]	R = rod-shape C = Centre T = Terminal E = Elliptical L = circular # = no detected	1 = S1-1 2 = S1-2 3 = 1M-1 4 = 1M-2 5 = 1M-3	6 = 1M-4 7 = 2M-1 8 = 2M-2 9 = 2M-3 10 = 2M-4	11 = 2M-5 12 = S2-1 13 = S2-2 14 = S2-3

(a) (b) (c)

Figure 1: Clear zone formation (a), Gram staining (b) and Endospore (c) from thermophilic bacteria

ISSN: 0975-8585

Amplification Using PCR of Gene Primer 16S rRNA

DNA isolation results shown in the quality and quantity by electrophoresis using agarose compared with λ DNA, the concentration of 50 ng / mL (Fig. 2a) Amplification product using the primers 27F and 1525R [10] produce fragments of, 1500 bp is visible from visualization electrophoresis (Fig. 2b). The visible difference in intensity of 14 isolates, this depends on the concentration of the resulting PCR product and the results of the PCR product is good enough for sequenced.

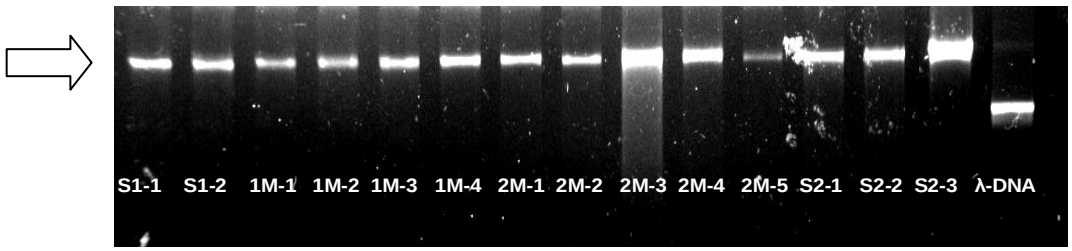

Figure 2a: Visualization electrophoresis agar rose of DNA thermophilic bacteria from 14 isolates

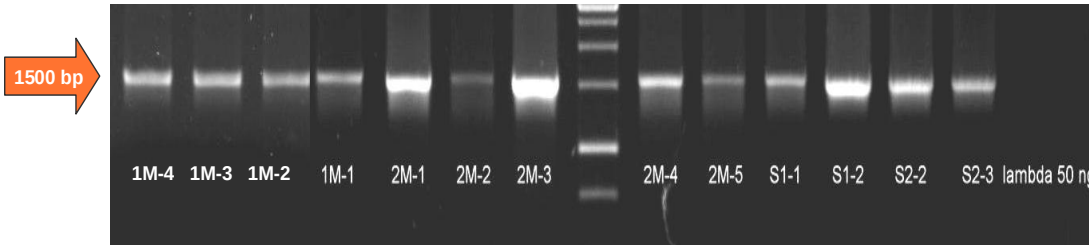

Figure 2b: Visualization electrophoresis agar rose of Product PCR (1500 bp) of thermophilic bacteria from 14 isolates

Sequence Analysis of DNA Thermophilic Bakeri, Based on 16SrRNA gene

BLAST analysis of DNA sequence thermophilic bacteria , compare the data sequences of thermophilic bacteria DNA sequencing results with DNA sequences available in the GenBank database online on the website http://www.ncbi.nlm.nih.gov/BLAST of NCBI (National Centre of Biotechnology Information) BLAST results of the 14 isolates of bacteria seen that almost 99.5% were Bacillus, it can be concluded that all isolates this is a group, the genus Bacillus and this is also supported by the results of phenotypic bacterial identification (Table 2). According Gomaa and Momtaz [11] 16S rRNA gene can be used to identify and characterize Bacillus, and pilogenetik tree can be used to see the kinship of species of a genus [12].

Tabel 2: Identification species of thermophilic bacteria base on gene 16S rRNA

Species	Total	Isolate Code	Similarity (%)\
Bacillus licheniformis	6 isolate	S1-1 S1-2 2M-1 2M-3 2M-5 S2-3	99.8 99.7 98,7 97,7 99,9 99,8
Bacillus subtilis	1 isolate	2M-2	99.7
Bacillus cereus	2 isolate	1M-1 1M-2	99,7 99,6
Bacillus sp.	5 isolate	1M-3 1M-4 2M-4 S2-1 S2-2	96,3 96,9 95,8 95,9 96,9

Phylogenetic Analysis of 14 Isolates of Thermophilic Bacteria

Nucleotide sequence obtained from the sequencing of each isolate thermophilic bacteria aligned (alignment) using CLUSTALX program, with sequences from the GenBank database by BLASTn results, to construct phylogenetic trees using MEGA5 program [13]. Phylogenetic tree can be used to see the kinship

ISSN: 0975-8585

between species based on similarities or differences in physical properties such as sequence or genetic sequence of DNA or amino acid (protein) bacteria that live in hot springs Rimbo Panti with various bacteria in the GenBank database. Based on the results of phylogenetic analysis and pylogenetic tree of 14 isolates obtained 3 Bacillus species and Bacillus sp. as in Table 2 and see figure 3 and 4.

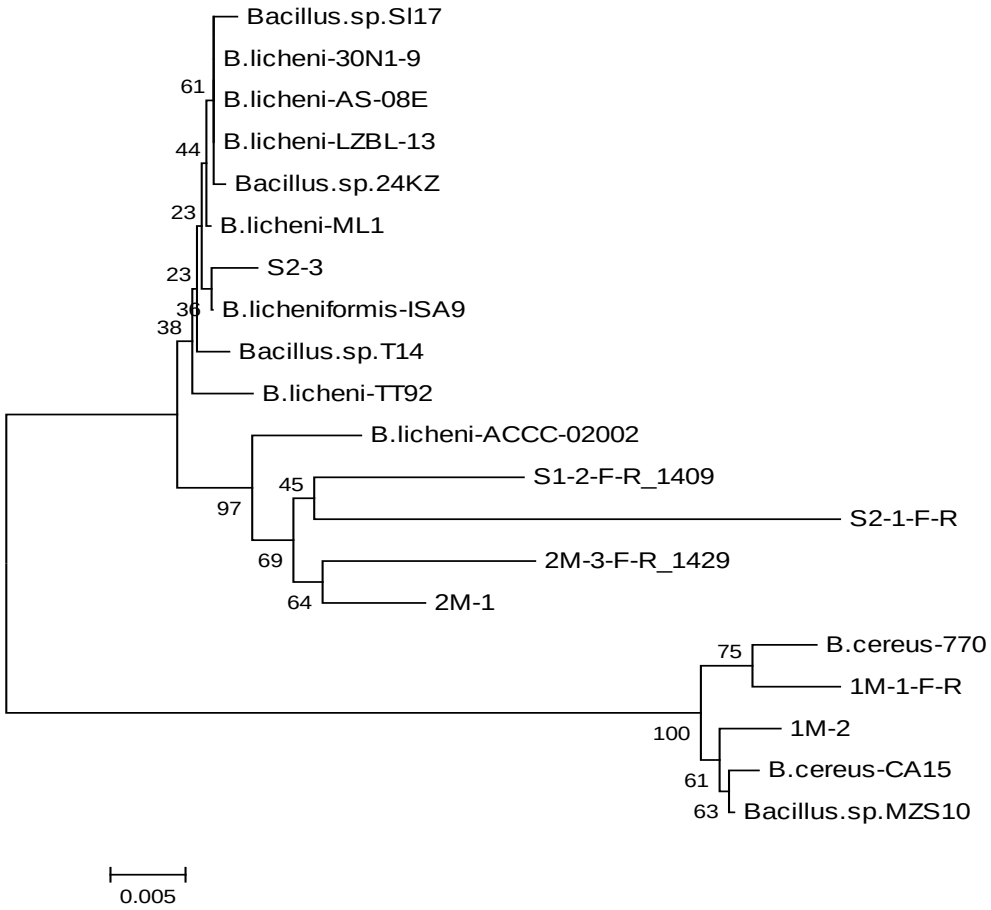

Figure 3: Phylogenetic tree isolat thermophilic bacterua which nucleotide sequence ±1500 bp

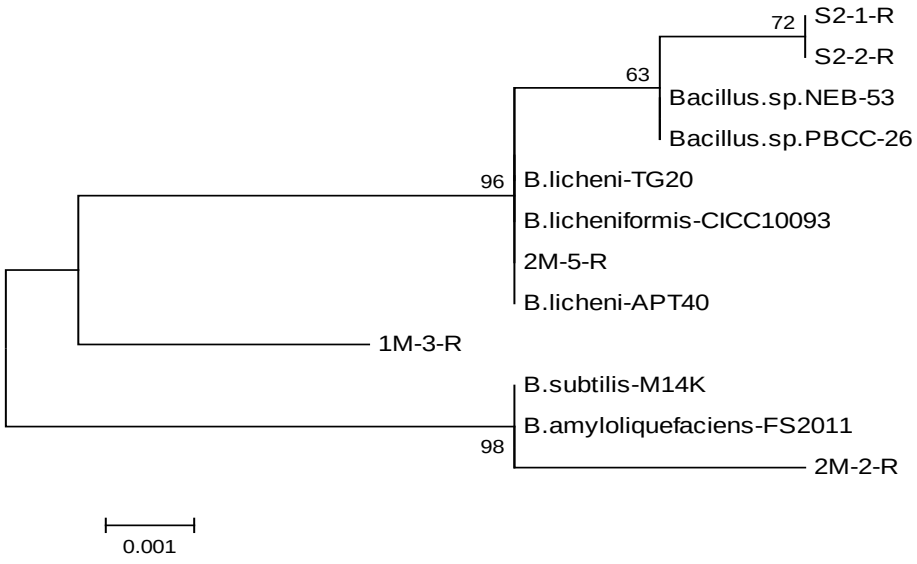

Figure 4: Phylogenetic tree isolat thermophilic bacterua which nucleotide sequence ≤800 bp

ISSN: 0975-8585

CONCLUSION

Thermophilic bacteria isolated from hot springs Rambo Panti obtained 14 isolates had cellulase activity (cellulolytic). The identification of 14 bacterial isolates belonging to the genus Bacillus and based on the results of phylogenetic analysis of 14 isolates obtained 3 *Bacillus* species as *Bacillus licheniformis*, *Bacillus cereus, bacillus subtilis* and *Bacillus* sp. S

REFERENCES

[1] Brock T. General Molecular, and Applied Microbiology John Will. New York, 1985.
[2] Pikuta EP and RB Hoover. Critical Reviews in Microbiology, 2007;33:183-209.
[3] Zhang YHP, Himmel ME, Mielenz JR. Biotechnol Adv 2006;24: 452-481
[4] Cava F, Hidalgo A, Berenguer J. Model Rev Extremoph 2009;13:213–231
[5] Elnasser Z, Maraqa A, Owais W, Khraisat A. The Internet J Microbiol 2008; 3: 2 .
[6] De Rosa M, Morana A, Riccio A, Gambacorta A, Trincone A, Incani O. Biosens Bioelectr 1994;9: 669–675.
[7] Armaini, Dharma A, Munaf E, Syukur S, Jamsari. Asian J Chem 2013;25.
[8] Bargey. The Manual of Determinative Bacteriology, 1974.
[9] Panosyan HH. Biolog J Armenia 2010;4(62).
[10] Baker GC, Smith JJ, Cowan DA. J Microbiol Meth 2003;55:541– 555
[11] Gomaa OM and OA. Momtaz. Arab J Biotech 2007;10:107-116.
[12] Wang Y and PY Qian. PLoS ONE 2009;4(10):7401.
[13] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, and Kumar S. Mol Biol Evol 2011;28:2731-2739.
[14] Vaseekaran SS. Balakumar and V Arasaratnam. Trop Agr Res 2010;22 (1): 1 - 11.