PROCEEDING

The 2nd International Seminar of Basic Science

Natural Science For Exploration The Sea-Island Resources

Ambon, May 31st 2016

Organized by

Faculty of Mathematics and Natural Science

Pattimura University

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The 2nd International Seminar of Basic Science

"Natural Science for Exploration The Sea-Island Resources"

Poka-Ambon, 31st May 2016

Mathematic and Natural Science Faculty
Universitas Pattimura
Ambon
2016

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ISBN : 978-602-97522-2-9

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2nd edition

Welcoming Address By The Organizing Committee

Today, We have to thank the The Almighty Allah SWT for the implementation of this international seminar. This is the second seminar about Basic Science in The Faculty of MIPA Pattimura University. The seminar under the title “Natural Sciences for Exploration the Sea-Island Resources” will be carried out on May 31st 2016 at Rectorate Building, Pattimura University. There are 200 participants from lecturers, research institute, students, and also there are 34 papers will be presented.

My special thanks refer to the rector of Pattimura University and the Dean of MIPA Faculty, Prof. Dr. Pieter Kakissina, S.Pd., M.Si. I also would like to express my deepest gratitude to Prof. Amanda Reichelt-Brushett, M.Sc., Ph.D. ; Kazuhiko Ishikawa, Ph.D. ; Nicolas Hubert, Ph.D. ; Prof. Dr. Kirbani Sri Brotopuspito ; Prof. Dr. Marjono, M.Phil. ; Gino V. Limon, M.Sc., Ph.D. as the keynote speakers.

The last, We hope this international seminar usefull for all of us, especially Mollucas People and very sorry if any mistake. Thank you very much.

Dr. La Eddy, M.Si.
Chairman of Organizing Committee

Opening Remarks
By Dean of Mathematic and Natural Sciences Faculty

I express my deepest gratitude to The Almighty God for every single blessing He provides us especially in the process of holding the seminar until publishing the proceeding of International Seminar in celebrating the 18th anniversary of MIPA Faculty, Pattimura University. The theme of the anniversary is under the title "Natural Sciences for Exploration the Sea-Island Resources". The reason of choosing this theme is that Maluku is one of five areas in Techno Park Marine in Indonesia. Furthermore, it is expected that this development can be means where the process of innovation, it is the conversion of science and technology into economic value can be worthwhile for public welfare especially coastal communities.

Having the second big variety of biological resources in the world, Indonesia is rich of its marine flora and fauna. These potential resources can be treated as high value products that demand by international market. Basic science of MIPA plays important role in developing the management of sustainable marine biological resources.

The scientific articles in this proceeding are the results of research and they are analyzed scientifically. It is expected that this proceeding can be valuable information in terms of developing science and technology for public welfare, especially people in Maluku.

My special thanks refer to all researchers and reviewers for your brilliant ideas in completing and publishing this proceeding. I also would like to express my gratefulness to the dies committee-anniversary of MIPA Faculty for your creativity and hard working in finishing this proceeding, God Bless you all.

Prof. Dr. Pieter Kakisina, S.Pd., M.Si.
Dean of Mathematic and Natural Sciences Faculty

ACKNOWLEDGMENT

The following personal and organization are greatfully

acknowledgment for supporting

"The 2nd International Seminar of Basic Science 2016"

Hotel Mutiara Ambon

Contents

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ISOLATION AND IDENTIFICATION OF LIPASE PRODUCING THERMOPHILIC BACTERIA FROM A HOT SPRING AT SERAM ISLAND, MOLUCCAS

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ABSTRACT

Lipases are enzyme that widely used in fat and oil processing, synthesis of fine chemichals and pharmaceuticals, food processing and lipid rich waste water treatment. Lipases from microbial sources prefered for biotechnological fields application due to their ability to catalyze a wide variety of hydrolitic and synthetic reaction. Thermophilic bacteria often possess enzyme that are stable and active at high temperature which are valuable for some biotechnological and industrial applications such as lipase enzyme for hydrolysis and esterification reaction. High temperature environment usally associated with volcanic phenomenas such as hot spring or geothermal vents. Isolat T 1.2 was thermophilic bacterium isolated from a hot spring at Seram island, which had temperature 89°C and pH 6.3. Isolation was conducted using Basal Salt Medium suplemented with olive oil, and Rhodamin B media. Comparison of 16SrDNA sequence to GenBank database using Basic Local Alignment Search Tools for nucleotides (BLASTn) showed that isolat T1.2 was closely related to *Geobacillus stearothermophilus* with 99% homology. The phylogenetic tree of 16SrRNA was constructed using Neighbour Joining Method and distance was estimated using Kimura's two parameter method with one hundred bootstrap replicates showed that the thermophilic isolat T1.2 is clustered to other members of *Geobacillus* and closely related to *Geobacillus stearothermophilus*.

Keywords : Thermophilic bacteria, lipase, *Geobacillus stearothermophilus*.

INTRODUCTION

Lipase (triacylglyserol acylhydrolase EC 3.1.1.3) catalyzed both hydrolysis and synthesis of long chain acylglycerol (Jaeger *et al*. 1999). Lipases are widely distributed in nature, being synthesized by plants, animals and microorganisms, mainly bacterial and fungal, are the most used as biocatalyst in biotechnological application and organic chemistry (Alvaro and Illanes, 2008). Catalytic process using lipase enzyme has several advantages compare to chemical catalysts, such as high specificity for substrate and catalyst target site, relatively simple and cheaper purification process, relatively high catalytic activity, environmentaly friendly and biodegradable (Devy *et al*, 2008). Lipases are used in industry to produce detergent, food processing, chemical synthesis and pharmacy, surfactan synthesis, oleochemical dan agrochemical industries (Vulfson, 1994). Lipases that stable at high temperature are used for processing lipids especially those have long chain unsaturated fatty

acids and need high temperature condition for their processing (Cho *et al*, 2000). Mollucas islands has many hydrothermal locations, home for various thermophilic microorganisms to live in. There are several methods to identified microorganisms, one of these methods using 16S rRNA gene. 16S rRNA gene was widely used for molecular identification, base on their characteristics such as it has constant function for live, universally distributed among living organisms and it has conserved sequence.

This research was aim to isolate and identified lipase producing thermophilic bacteria from a hot spring at Seram island, Mollucas.

MATERIALS AND METHODS

Soil and water samples

Soil and water samples were collected from a hot spring at Seram Island, Mollucas.

Isolation of Lipase Producing Thermophilic Bacteria

Lipase producing thermophilic bacteria isolation was done by inoculated 1 loup of water and soil sample to Basal Salt Solution media supplemented with yeast extract and olive oil, and incubated at 60°C for 48 hours with shaking. After incubation, culture was spreaded on Rhodamin B media (Kouker & Jaeger, 1988) and incubated at 60°C for 24 hours. Bacterial colonies with lipase activity will show orange fluorescent halos around bacterial colonies visible upon UV irradiation.

Lipase Producing Bacteria Identification

DNA extraction was conducted by colony PCR method (Packeiser *et al*, 2013). Amplification of 16S rDNA was performed by PCR using universal primer pair (63f and 1387r) specific for bacterial domain (Marchesi et al, 1998). PCR conditions was set as follows : pre PCR (94°C, 7 min), followed by 34 cycle of denaturation (94°C, 30 s), annealing (56°C, 30 s), elongation (72°C, 1 min, 30 s) and finally 72°C, 7 min). PCR products were analyzed using 1,5% agarose. Gel was soaked in ethidium bromide solution for 15 minutes and rinsed with water, the results were detected using a UV transilluminator.

DNA sequencing and Phylogenetic Analysis

The amplified DNA were sequenced (AB Applied Biosystems Hitachi 3130 Genetic Analyzer). The sequenced data were processed using Bioedit programme. The homology of 16S rDNA sequenced was searched using BLASTN at the NCBI website and the references sequence was obtained from the GenBank (www.ncbi.nlm.nih.gov). Construction of phylogenetic tree was done using neighbour-joining tree method (NJT) on MEGA 5.0 software (Tamura *et al*, 2011), the distance was predicted using Kimura two parameter method. Strength of internal branches of the phylogenetic tree was tested with boostrap analysis using 100 replications.

RESULTS AND DISCUSSION

Sample of water and soil was taken from a hot spring at Seram Island, Mollucas, which has temperature 89°C and pH 6.3. Some microorganism have been known to lived and grow at high temperature environments and have been grouped as thermophilic microorganisms (temperature range 45°C to 80°C) and hyperthermophilic microorganism (temperature above

80°C until the highest temperature known to support microorganism growth which was 113°C) (Stetter 1999, Dehouck *et al* 2008). One colony of bacteria, which have been named isolate T 1.2, that shown lipase activity on rhodamin B media (Figure 1) was isolated for further analysis. Isolate T 1.2 had crude extract lipase enzyme activity optimum at 80°C, and still retain 47% of its activity at 90°C however there was no enzyme activity detected at 50°C which mean the activity of lipase from isolate T I.2 is thermophilic obligate (Data not shown).

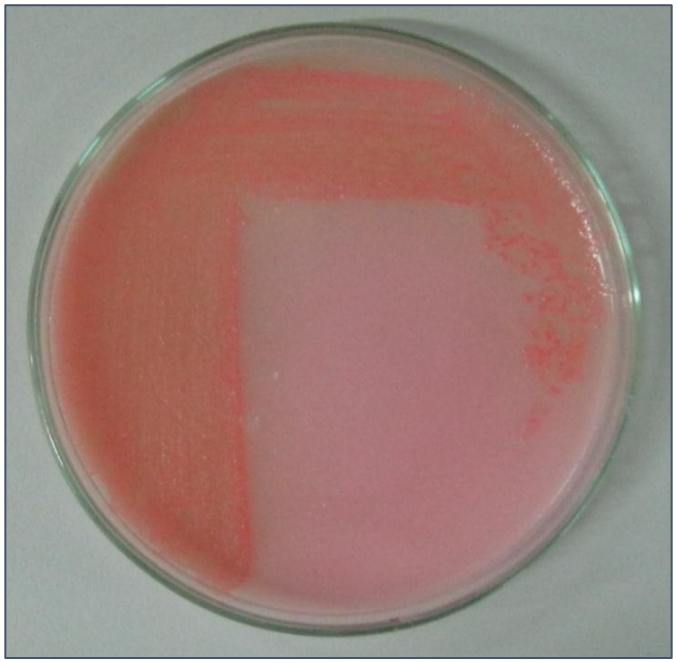

Figure 1. Isolat T I.2 was grown on rhodamin B media

Amplification of 16S rRNA gene from isolat T I.2 using universal primer pair (63f and 1387r) specific for bacterial domain produce DNA fragmen with size in the range of 1200 bp to 1500 bp, this result show that 16S rRNA gene from isolat T I.2 was able to be amplified well (Figure 2).

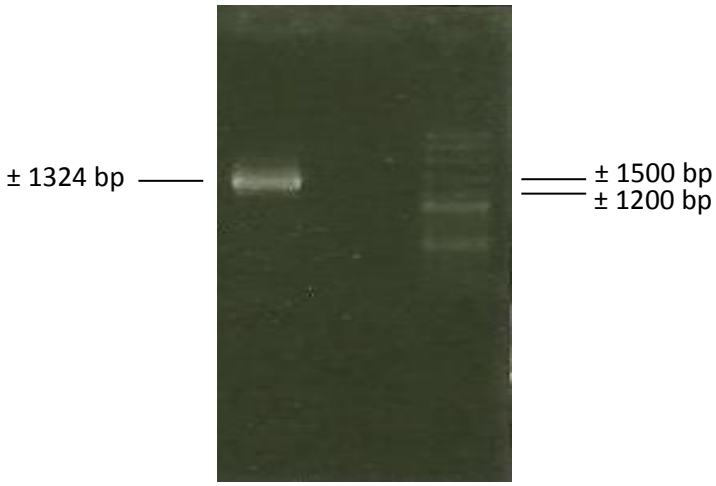

Figure 2. Agarose gel electrophoresis analysis of amplified 16S rRNA gene from isolat T I.2 using 63f and 1387r primers (S : Gene fragment of 16S rRNA from Isolat T I.2, M : Molecular wight markers)

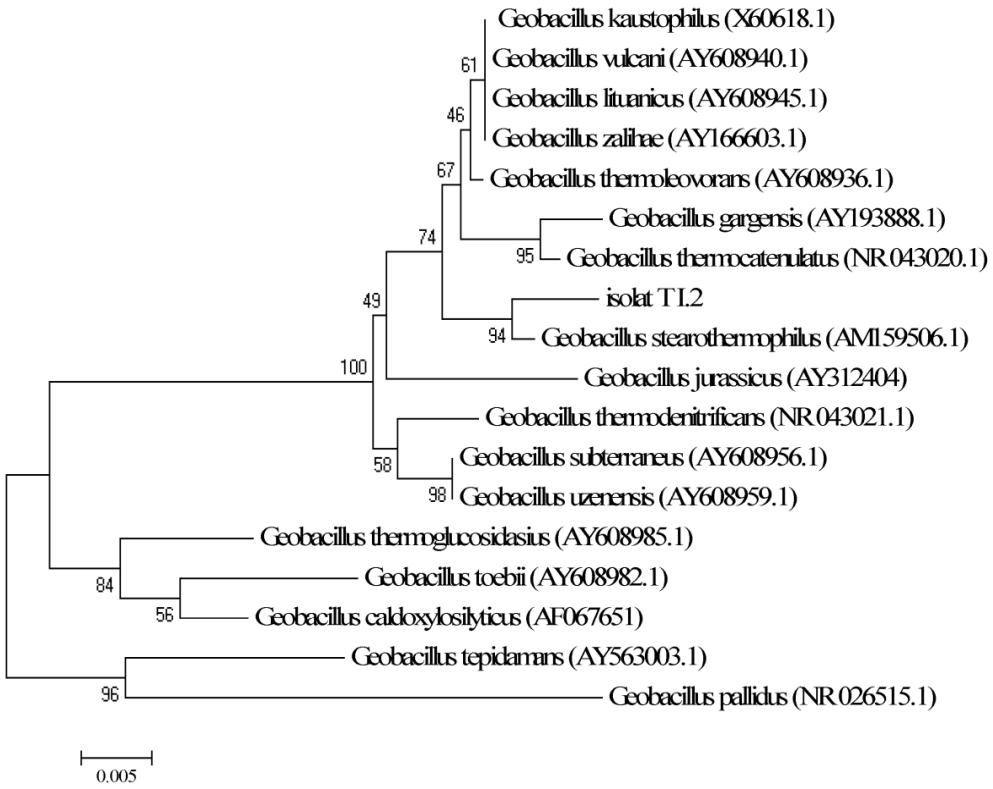

Figure 3. Phylogenetic position of isolat T I.2 among *Geobacillus* species**.**

Amplified 16S rRNA fragment from isolat T I.2 was then sequenced and the result was compared to the references sequences obtained from the GenBank using BLASTN, the sequence of 16S rRNA of isolat T I.2 was also used for constructing phylogenetic tree. BLASTN result showed that isolat T I.2 have 99% similiarity to *Geobacillus stearothermophilus* (Data not shown). Taxonomy hierarchy of isolate T I.2 base on ribosomal data base project (Cole *et al* 2005) :

domain *Bacteria*
phylum *Firmicutes*
class *Bacillus*
order *Bacillales*
family *Bacillaceae*
genus *Geobacillus*

Phylogenetic tree base on 16S rRNA genes show that isolat T 1.2 is clustered among members of *Geobacillus* genera, and have position closest to *Geobacillus stearothermophilus* (Figure 3). *Geobacillus stearothermophilus* belongs to *Geobacillus* genera, a genera that consists of obligate thermophilic bacterial species previously known as group 5 of *Bacillus* genera (Nazina *et al*. 2001).

CONCLUSION

We have isolated lipase producing thermophilic bacteria from a hot spring at Seram island, Mollucas which had temperature 89°C and pH 6.3. Identification base on comparison of 16SrRNA gene sequences from isolat T I.2 to GenBank database shown that isolat T I.2 have 99% homology to *Geobacillus stearothermophilus.* Analysis of phylogenetic tree of 16SrRNA gene showed that the thermophilic isolat T I.2 is clustered to other members of *Geobacillus* and closely related to *Geobacillus stearothermophilus*.

ACKNOWLEDGMENT

The authors would like to thank PT. Wilmar Benih Indonesia for financial support as well as permission to use the reasearch facility.

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Design By : Hanter Olong