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"Science Enhancement for Developing Countries"

FACULTY OF MATHEMATICS AND NATURAL SCIENCES

HASANUDDIN UNIVERSITY

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Publisher: Fakultas Matematika dan Ilmu Pengetahuan Alam UNHAS

Address: Jl. Perintis Kemerdekaan KM 10 Tamalanrea 90245 Makassar

Tlp (0411) 586016, Fax (0411) 588551

Email: ahaddade@fmipa.unhas.ac.id

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PREFACE

Makassar city, the capital of South Sulawesi province known as one of the biggest cities in Indonesia and also having Hasanuddin University, the biggest university in eastern part of Indonesia, has plenty of natural resources and human resources. Having a strategic position at the center point of Indonesia, Makassar has been developing very rapidly, and has been contributing to the regional, national and even international economic development. Given this, science can play important roles and therefore is needed to support rapid development in various sectors.

With regard to this, cooperates with Ministry of Environment Indonesia, Atmospheric and Ocean Research Institute (AORI) Japan, University of Kebangsaan Malaysia (UKM), Alfred Wagener Institute (AWI) Germany, Queensland University of Technology (QUT) and Flinders University Australia, Faculty of Mathematics and Natural Sciences Hasanuddin University carried out "The First International Conference on Science (ICOS-1)" on November 19-20, 2014, in Hotel Clarion Makassar. The theme of ICOS-1 is "Science Enhancement for Developing Countries". The conference attended by two hundred participants and came from Asia (Japan, Malaysia, Indonesia), Australia, and Europe.

There are approximately 97 research articles for oral presentations and 16 poster presentations, ranging from Biology, Statistics, Mathematics, Chemistry, Physics, Geophysics, Computer Science and Environmental Science. Of the 113 papers, there are approximately 79 papers were selected to be published in the proceedings of the ICOS-1 through the peer review process.

With regard to the delivery of the ICOS-1 in 2014 and the completion of the proceedings ICOS-1, 2014, allow us to thanks to: the authors for providing the content of the program, the conference participants who came from several public and private universities, the program committee and the senior program committee, who worked very hard in reviewing papers and providing feedback for authors to be included in the Proceedings of ICOS-1, 2014, the hosting organisation Hasanuddin University, our keynote and invited talk presentations including Ir. Muh Ilham Malik M.Sc, from Ministry of Environment Indonesia, Prof Koji Inoue from AORI Japan, Prof Mohammad B Kassim from UKM Malaysia, Dr.rer.nat Dominik Kneer from AWI Germany, Prof Dadang A. Suriamihardja and Prof Alfian Noor from Hasanuddin University, Prof Kerrie Mengersen from QUT and Dr. Darfiana Nur from Flinders University, Australia.

Hopefully is of benefit to all readers.

Yours faithfuly,
Prof Dr. Hanapi Usman M.S
Dean of Faculty Mathematics and Natural Sciences
Hasanuddin University

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MOLECULAR IDENTIFICATION OF BACTERIAL SIMBIONT MACROALGAE *Sargassum polycystum* PRODUCING ENZYMES L-ASPARAGINASE

Mashuri Masri

Biology Department, Sciene and Technology Faculty, Islamic state University of Alauddin Makassar, South Sulawesi, Indonesia 92113. mashuriuin@gmail.com

Abstract

Molecular Identification of bacterial Simbiont Macroalgae Sargassum polycystum Producing Enzymes L-Asparaginase were isolated from Barrang Lompo Island South Sulawesi in 2013. Among them three bacterial strains exhibiting glutaminase free L-asparaginase activity were identified as Pseudomonas putida, Enterobacter asburine dan Marinobacter arcticus on the basis of 16S rRNA gene sequencing. The Highest Max Score 1358 and Query Cover 98 % were Pseudomonas putida furthermore identified as bacterial L-asparaginase activity from Sargassum sp. Pseudomonas putida has Identities 756/765 (99%) with 778 bp Query length, Query cover 98% and Gaps 5/765 (0%).

Keywords : *L-Asparaginase, Pseudomonas putida, bacterial Simbiont Macroalgae Sargassum polycystum.*

1. INTRODUCTION

Interaction of secondary metabolites produced by marine bacteria associated with probable cause bacteria induced to produce a specific bioactive metabolites. According Nofiani (2008), these bacteria have the ability similar to the host to produce bioactive compounds.

Most algae that have different bioactivity ranging from antibacterial, antifungal, anticancer and etc were a group of brown algae, mainly from *Sargassum* and *Turbinaria* group. Some types of brown algae, among others *Sargassum cinereum*, *Sargassum hemiphyllum*, *Sargassum polycystum*, *Sargassum echinocarpum* and *Turbinaria decurrens* were found in the waters of South Sulawesi (Rashid, 2001).

L-Asparaginase is one component of nutrition for cancer cells. Giving of L-Asparaginase in cancer cells can decompose L-Asparagine which is expected to inhibit the growth of these cells. L-Asparaginase of great benefit in cancer therapy (E-Moharam, et al, 2010), one of them is a cervical cancer cells.

2. METHODOLOGY

2.1 DNA Ekstraction

DNA extraction is done in accordance with the procedures of QIAamp® DNA kit (QIAGEN) A total of 140 mL of bacterial swab (sample) mixed into 1.5

ml micro-tubes containing 560 mL of buffer AVL then vortex for 1 minute and incubated at room temperature for 10 minutes. Tubes were centrifuged for a while to reduce fluid tube attached to the lid, then added 560 mL of absolute ethanol into the tube and vortex 1 minute. A total of 630 mL mixture inserted into the column and then centrifuged at 8000 rpm for 1 minute in orther the solution out of the column and into the reservoir tube and then discarded, while the DNA remained in the column. Column washed with wash solution containing ethanol that is AW1 and AW2 respectively by adding 500 mL and centrifuge at 8000 rpm for 1 minute. To remove excess wash solution that may remain in the column, centrifuge at 12,000 rpm for 1 minute. Reservoir tube was then replaced with 1.5 ml sterile eppendorf tube. To release the DNA trapped in the column added 60 mL elution solution AVE (AVE buffer) and then incubated at 50 º C for 1 minute. Centrifugation at 12,000 rpm for 1 minute, liquid containing the DNA deposited in a sterile tube (Samaila, A., 2013).

2.2 DNA Amplification

A master mix containing 1 mL U1, U2 1 mL, 5 mL 10X buffer, 2 mL 25 mM $MgCl_2$, 1 mL of 10 mM dNTPs, 0.25 mL of hot start, 29.75 mL H_2O and 10 mL of DNA. Dissolving 10 mL of supernatant and 40 mL master mix so that the total volume of 50 uL sample then put in a PCR machine. PCR cycle stages, namely denaturation, annealing and extension, carried out at a temperature of 94ºC for 30 seconds, 50ºC for 30 seconds and 72ºC for 1 minute 30 seconds for the second stage (stage 35 cycles to 30 cycles for the first and second stage). PCR cycle begins with a pre-denaturation step at 94ºC for 5 minutes and ended with a final extension step at 72ºC for 5 minutes (Samaila, A., 2013).

2.3 Gel Agarose Electroforesis

A 2% agarose was used in this study. A total of 5 mL ethidium bromide solution (10 mg / mL) was added to the agarose gel, TAE solution was then added to the electrophoresis tank to cover the upper surface of the gel. Into each well, put 5 mL of PCR product was mixed with 1 mL after loading dye solution (40% sucrose, 0.25% bromophenolblue). In one of the wells included 5 mL (0.5 mg) of DNA molecular weight marker XIV (100 bp) as a DNA size standard. Electrophoresis device is run by giving power to the voltage to 80 volts (V) for 30 minutes. Once the process is complete agarose gel electrophoresis, the gel was entered in a Gel Doc XR, Biorad, USA (Samaila, A., 2013).

2.4 Sequencing

RNA forward samples were sent to MacroGen Korea for sequencing. Nucleotides were inserted into the BLAST program.

3. Result and Discussion

The similarity with the nucleotide sequences derived from other bacteria can be analyzed by BLASTN program on online sites at http://www.ncbi.nlm.nih.gov/blast.

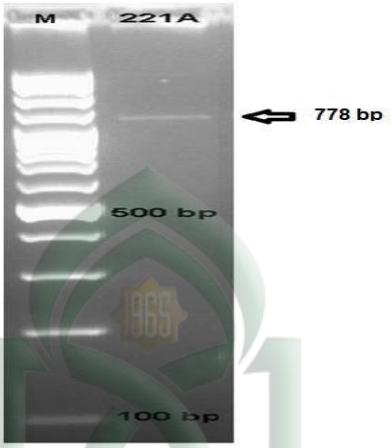

Fig 1. Agarose gel electrophoresis of 16S Ribosomal RNA from isolates of bacterial symbionts macroalgae *Sargassum polycystum.*

16S Ribosomal RNA gene expressed by SBJCT while the sample is expressed with QUERY. There are 778 bp Query length. The length of the 16S Ribosomal RNA gene was used for 1419 bp with a range of 518 bp to 1277 bp. The use of 518 bp to 1277 bp by the system because in that section is the closest approximation to the nucleotide sequence of the sample with 98% cover query value (Fig 2 and Fig 3).

Molecular identification of the identification results is *Pseudomonas putida* strain E16 16 ribosomal RNA, Accession KC820813.1 (fig 2). The same result research with the study of El-Bessoumy et al., (2004) who identify bacteria producing the enzyme L-Asparaginase from Pseudomonas aeruginosa 50071.

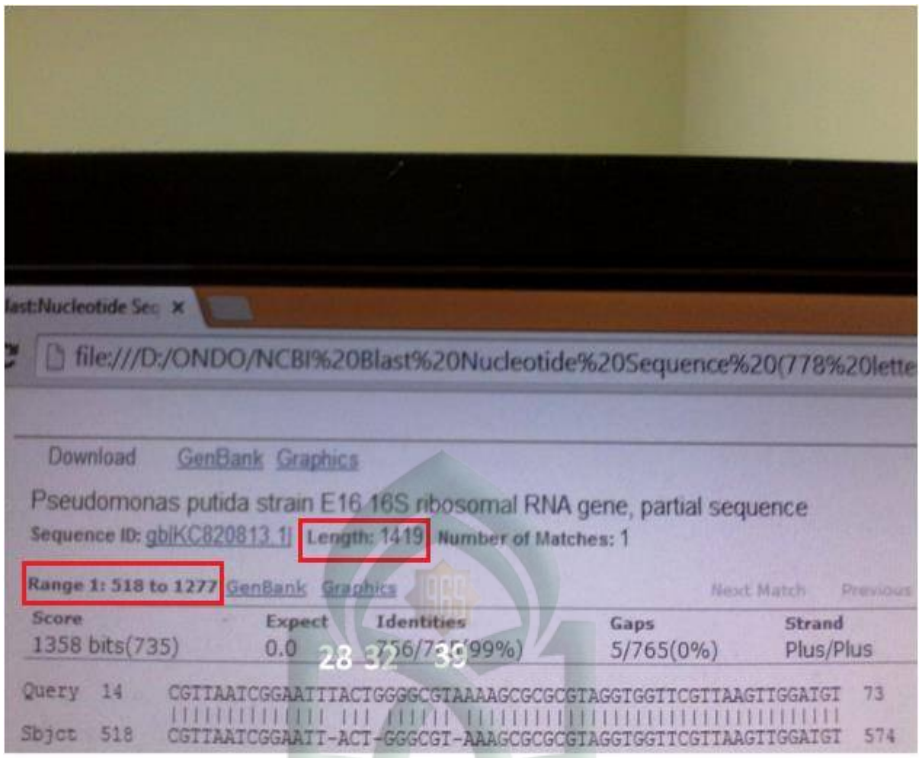

Fig 2. Psedomonas putida strain E16 16S ribosomal RNA gene, partial sequence

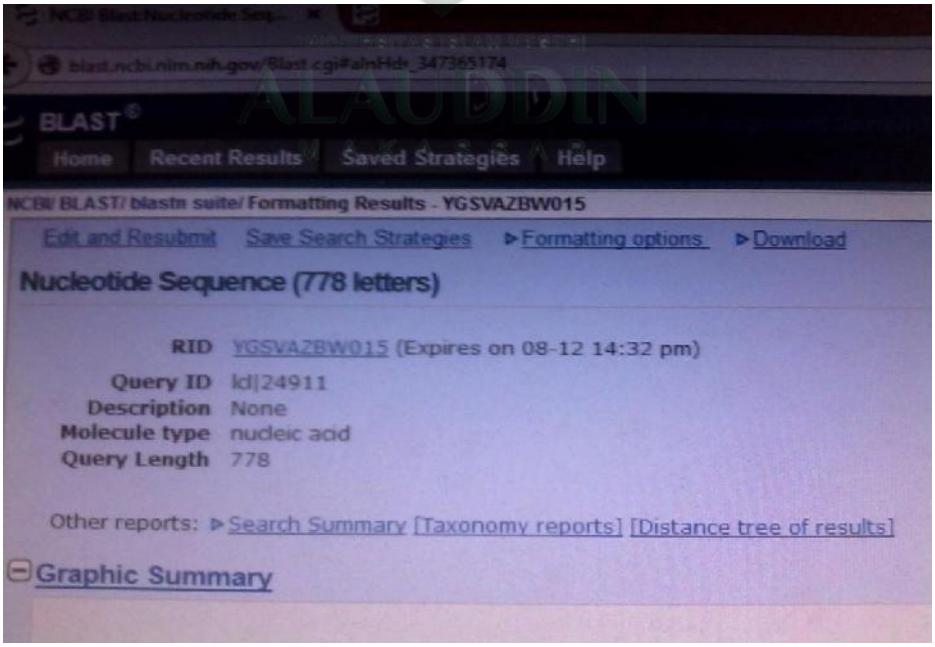

Fig 3. 778 bp query length.

1 bp
GCAAGAGATACCCCGTTAATCGGAATTTACTGGGGCGTAAAAG
CGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAAC
CTGGGAACTGCATCCAAAACTGGCGAGCTAGAGTACGGTAGAGGGTG
GTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACA
CCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCG
AAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCA
GCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAA
ACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
TTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAG
AACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGC
TGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGT
AACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCAC
TCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT
CAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGG
TCAGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCTCATAAA
ACCGATTCGTATTCCCAGAT
778 bp

Fig 4. Nucleutyde base sequencing

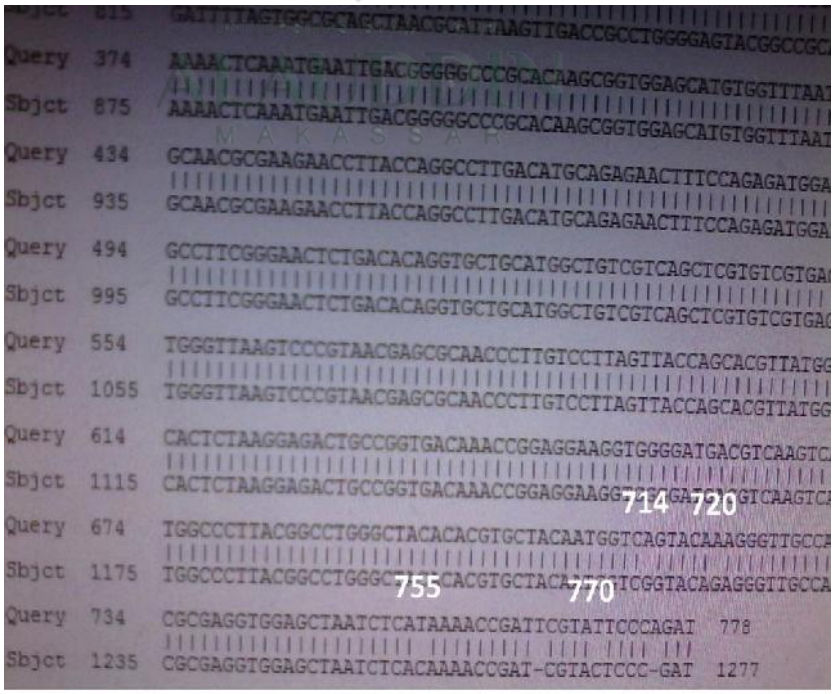

Fig 5. 4 bp experiencing substitution.

Identities of 756/765 (99%). This means that there are 756 bp in common between the query and Sbjct of 765 bp. There are 9 differences from 5 bp experiencing insertion (named *gaps*) and 4 bp experiencing substitution.

This four substitution seen in figure 5 that the Query 714 bp, 720 bp, 755 bp, 770 bp. The first substitution occurs in the Query 714 bp in the form of A but in the form of G in Sbjct 1215 bp. The second substitution occurs in the query 720 bp in the form of A but in the form of G in Sbjct 1221 bp. The third substitution occurs in the query 755 bp in the form of T but in the form of C in Sbjct 1256 bp. The fourth sub stitution occurs in the query 770 bp in the form of T but in the form of C in Sbjct 1270 bp.

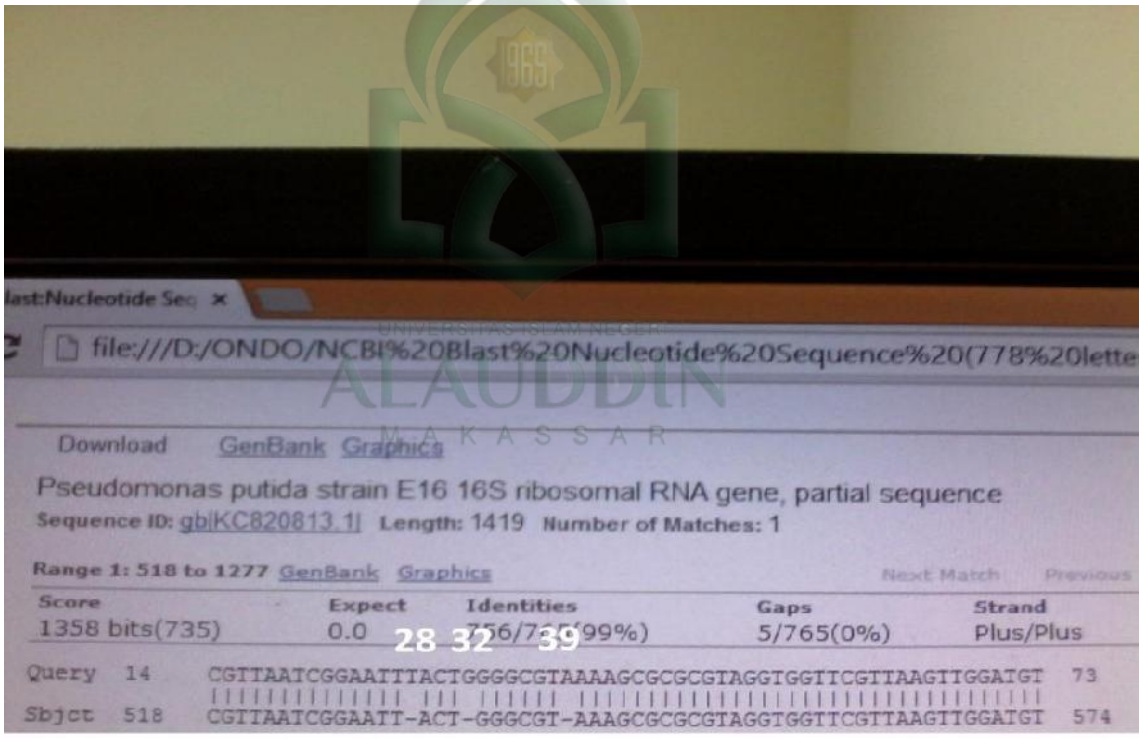

Fig 6. 3 bp experiencing insertion

Fig 7. 2 bp experiencing insertion

Gaps of 5/765 (0%). It is intended that the queries 765 bp long and 5 are gaps with Sbjct, ie, on the order of 28 bp, 32 bp, 39 bp, 765 bp and 775 bp that occurs due to insertion at the Query (contained in the query but not found in sbjct).

The total insertion are five, there are three insertions in figure 6, ie 28 bp, 32 bp, 39 bp and two insertions in figure 7, ie 765 bp and 775 bp.

There are three possibilities of bacteria ie *Pseudomonas putida* with Max score 1358. QC (query cover) = 98%. *Marinobacter arcticus* with Max score 1356. QC = 97% and *Enterobacter asburiae* with Max Score 1356. QC = 97%. From these results determined that the strain of *Pseudomonas putida* E16 16S ribosomal RNA gene (Accession KC820813.1) is a bacterial symbiont of macro algae *Sargassum policystum*.

4. CONCLUSION

Pseudomonas putida E16 16S ribosomal RNA gene (Accession KC820813.1) is a bacterial symbiont of macro algae *Sargassum policystum*.

5. BIBLIOGRAPHY

[1] E-Moharam, M., Gamal-Eldeen, A.M., dan El-Sayed, S.T., 2010, *Production, Immobilization and Anti Tumor Activity of L – Asparaginase of Bacillus sp R36*, Journal of American Science, Vol. 6.

[2] El-Bessoumy, A.A., Sarhan, M., dan Mansour, J., 2004, *Production, Isolation, and Purification of L – Asparaginase from Pseudomonas Aeruginosa 50071 Using Solid state Fermentation*, Journal of Biochemistry and Molecular Biology, Vol. 37.

[3] Nofiani, R., 2008, *Urgensi dan Mekanisme Biosintesis Metabolit Sekunder Mikroba Laut*.

[4] Rasyid, A., 2001, *Potensi Sargassum Asal Perairan Kepulauan Spermonde sebagai Bahan Baku Alginat*, Kandidat Peneliti Puslitbang Oseanologi, LIPI, Jakarta.

[5] Samaila, A., 2013, Identifikasi Bakteri di Udara Ruang Perawatan Intensif Rumah Sakit Wahidin Sudirohusodo dengan Universal Primer dan Sekuensing, TESIS S2 Biomedik Mikrobiologi, Kedokteran, UNHAS, Makassar.

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