

International Student **C**onference on **A**dvanced **S**cience and **T**echnology

The 8th ICAST 2013 Kumamoto

December 12-13, 2013
Kumamoto University, Japan

Research Session

Mathematics

Physics

Chemistry

Earth and Environments

Biological Sciences

Applied Chemistry and Biochemistry

Materials Science and Engineering

Mechanical System Engineering

Computer Science and Electrical Engineering

Civil and Environmental Engineering

Architecture

General Session

Energy environment and natural disasters

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Graduate School of Science and Technology ◀ Kumamoto University

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Conference Program

December 12, 2013

8:30 – 9:20	Registration (100th Anniversary Memorial Hall)
9:30 – 9:40	Opening Address Isao Taniguchi (President of Kumamoto University)
9:40 – 9:50	Welcome Address Kazuki Takashima (Dean of GSST, Kumamoto University)
9:50 – 10:30	Keynote Speech Tsuyoshi Usagawa (Professor of GSST, Kumamoto University)

Reports on international experiences by GSST students

10:30 – 10:45	Shojiro Matsuki (Dept of Civil and Environment Engineering)
10:45 – 11:00	Ryosuke Sueda (Dept of Computer Science and Electrical Engineering)
11:00 – 11:15	Retyce Ivan Herve Dodji T Amoussou (Dept of New Frontier Sciences)
11:15 – 11:30	Ranipet Hafeez Basha (Dept of Advanced Mechanical Systems)
11:30 – 11:45	Wei-Cherng Cheng (Dept of Computer Science and Electrical Engineering)

13:00 – 15:00	Oral Session I (Room A) 5-1, 5-2, 5-3, 5-4, 5-5, 5-6, 5-7, 5-8, 9-1, 9-2
	Oral Session II (Room B) 1-1, 2-1, 2-2, 2-3, 2-4, 6-1, 6-2, 6-3, 6-4, 6-5
	Oral Session III (Room C) 4-1, 4-2, 4-3, 4-4, 4-5, 4-6, 10-1, 10-2, 10-3, 10-4
	Oral Session IV (Room D) 12-1, 12-2, 12-3, 12-4, 12-5, 12-6, 12-7, 12-8, 12-9, 12-10, 12-11
15:00 – 15:30	Break

15:30 – 17:20	Oral Session I (Room A) 9-3, 9-4, 9-5, 9-6, 9-7, 9-8, 9-9
	Oral Session II (Room B) 6-6, 6-7, 6-8, 3-1, 3-2, 3-3, 3-4, 3-5
	Oral Session III (Room C) 10-5, 10-6, 10-7, 10-8, 10-9, 10-10, 10-11, 10-12
	Oral Session IV (Room D) 14-1, 14-2, 14-3, 14-4, 14-5, 14-6, 14-7, 14-8

18:15 – 20:00	Student Exchange Party (FORICO)
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December 13, 2013

9:00 – 10:40	Oral Session I (Room A) 7-1, 7-2, 7-3, 7-4, 7-5, 7-6, 7-7, 7-8
	Oral Session II (Room B) 3-6, 3-7, 3-8, 3-9, 3-10, 3-11, 3-12, 3-13
	Oral Session III (Room C) 10-13, 10-14, 10-15, 10-16, 10-17, 10-18, 10-19, 11-1
	Oral Session IV (Room D) 13-1, 13-2, 13-3, 13-4, 13-5, 13-6, 13-7, 13-8
10:40 – 11:00	Break
11:00 – 12:30	Oral Session I (Room A) 8-1, 8-2, 8-3, 8-4, 8-5
	Oral Session II (Room B) 3-14, 3-15, 3-16, 3-17, 3-18
	Oral Session III (Room C) 11-2, 11-3, 11-4, 11-5, 11-6, 11-7, 11-8
	Oral Session IV (Room D) 13-9, 13-10, 13-11, 13-12, 13-13, 13-14
Poster Session (100th Anniversary Memorial Hall)	
13:30 – 14:30	Odd number poster presentation
14:30 – 15:30	Even number poster presentation
15:30	Closing

December 14, 2013 Excursion to Mt. Aso

Room A: Meeting Room A, 2nd floor, Faculty of Engineering Building No.1

Room B: Lecture Room, 2nd floor, Academic Commons Kurokami-1

Room C: Meeting Room 204, 2nd floor, Multidiscipline Laboratory Building

Room D: Seminar Room, 2nd floor, Graduate School of Science and Technology Research Building

Research Session

1. Mathematics
2. Physics
3. Chemistry
4. Earth and Environments
5. Biological Sciences
6. Applied Chemistry and Biochemistry
7. Materials Science and Engineering
8. Mechanical System Engineering
9. Computer Science and Electrical Engineering
10. Civil and Environmental Engineering
11. Architecture

General Session

12. Energy
13. Environment
14. Natural Disaster

Antimicrobial activity of bacteria associated with the tunicate *Ascidia ornata*

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Abstract- The tunicate *Ascidia ornata* has done screening of bacteria that can produce antimicrobial compounds. The aim of the study was to find out characteristics of antimicrobial activity of *A. ornata* associated bacteria extracts. The species tunicate from the island in Doreri Bay Manokwari, West Papua. Bacteria that associated with *Ascidia ornata* were then isolated on SWC medium. From 6 isolates from *A. ornata* have 6 isolates and they are different type. From 6 isolates just 1 isolates can identified enteropathogenic *Escherichia coli* K-11 (EPEC K-11) and *Candida albicans*. The results showed that bacterium is gram negative bacterium and it showed 99% homology with *Escherichia coli* strain LMG 2693. The optimum biomass synthesis was obtained after 48 h of incubation at agitation speeds at 120 rpm, initial fermentation pH at 7.0, incubation temperature at 30°C, using glucose as a source of carbon concentration of 0.1 % (w/v.)

Keywords- Isolation, screening of antimicrobial activity, 16S rDNA sequencing, marine organism, marine bacteria.

I. INTRODUCTION

Indonesia marine resource potential as providers of natural materials for the pharmaceutical industry is ascidians and tunicates. Tunicates is a rich biota of bioactive compounds that can be used as a drug or medicinal substance. Bioactive components that exist in ascidians include antiviral, immunosuppressants, and cytotoxic agents (Garateix, 2004).

Marine bacteria are a source of new bioactive compounds that concern today. Bacteria can produce bioactive compounds for a variety of biological activities, such as antimicrobial. Production of antimicrobial compounds is one way bacteria adapt to the environment in an effort to defend against bacteria (Penesyan *et al.*, 2010). Based on the above research was conducted isolation of marine bacteria contained in tunicates *Ascidia ornata*. Parameters observed antimicrobial activity was also observed screening of their antimicrobial activity. Identification of one of the best marine bacterial strains using various techniques and optimization of the antimicrobial productivity.

II. MATERIAL AND METHODS

A. Isolation of bacteria from *Ascidia ornata*

Sampling site is located on Lemon Island in the Doreri Bay Manokwari, West Papua. Research station is an artificial reef slope at a depth of 3-15 meters. The size of *Ascidia ornata* is five centimeters in length were taken at a depth of 3-15 meters at high tide in the waters of Manokwari. Surface of tunicates were rinsed with sterile seawater. For the isolation of bacteria taken from tunicates the first taken from the entry

chiffon, chiffon out and the center tunic by using a sterile swab and then homogenized in 10 ml PBS. Suspension homogenization result suspension diluted serially in PBS. Suspension each dilution rate of 100 µl poured and leveled in the media Sea Water Complet (SWC) with the composition of the media 5 g / l bacto peptone, 1 g / l extract yeast and 3 ml / l glycerol, and incubated at 30°C for 1-2 days. Each colony isolates purified by repeatedly scraping the quadrant in SWC medium to obtain single colonies were pure. The isolated strain was identified morphologically (shape, gram staining, spore staining and motility), biochemically Microbact Kit 12A and 12B.

B. Screening of isolates for antimicrobial activity

In primary screening, determination of the antimicrobial activity of pure isolates was done by the method of double layer (Cappucino and Sherman, 2001) using three microbial test strain. They were methicillin-resistant *Staphylococcus aureus* (MRSA), enteropathogenic *Escherichia coli* K-11 (EPEC K-11) and *Candida albicans* (Clinical isolate). The resulting bioactive antimicrobial compounds indicated by the formation of clear zones around colonies of pure isolates. For microbial testing of *Candida albicans* media used were Potato Dextrose Agar (PDA).

C. Identification of bacterial strains

The bacteria with wide antimicrobial spectrum were identified to the species level by PCR amplification of the 16S-rDNA gene, BLAST analysis and comparison with sequences in the GenBank nucleotide database. Specifically, the 16S-rDNA using universal forward primer 16F27 (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse primer 16R1525 (5'-AAG GAG GTG ATC CAG CCG CA-3'). The PCR products were purified by QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). Sequence data were analyzed by comparison with 16S rRNA genes in the GenBank database. The nearest relative of the organism was obtained by BLAST searches and aligned with close relatives using the Clustal W software. A phylogenetic tree was constructed with MEGA version 5.0 using neighbor-joining method (Tamura *et al.*, 2007).

D. Fermentation for Antimicrobial

A primary The effects of agitation speeds 120 rpm, incubation temperature 30°C, initial pH 7 inoculum size 10%, and carbon sources 0,1 % (w/v). About 10% (v/v) of an overnight preculture of the active marine isolate was added in separate flasks of 2 broth medium. Growth of the bacterial cells of the marine isolate was evaluated by measuring of the optical density of the culture. The sample was diluted with 10-fold and measured by spectrometer (UV.

Spectrophotometer) at 620 nm.

E. Results and discussion

Screening of isolates for Antimicrobial activity

Among six marine strains isolated from the tunicate *Ascidia ornate*, one showed antimicrobial activity to at least one terrestrial microorganism. Isolat AOSM2 was able to produce the activity of the antimicrobial substance against Enteropathogenic *Escherichia coli* K-11 (EPEC K-11) and *Candida albicans*. Therefore, isolat AOSM2 was chosen for identification and further physiological studies.

F. Identification of strain AOSM2

The strain AOSM2 showed is gram negative, rod, non motil, non spora, the biochemical characteristic results are Lysine (+), Ornithine(+), H₂S(-), Glucose(+), Mannitol(+), Xylose(+), ONPG(+), Indole(-), Urease(+), V-P(+), Citrate(+) and TDA(+). The 16S-rDNA sequence analysis indicated that AOSM2 was closely related to the member of genus *Enterobacter*. The highest sequence similarity values 99 % was *Enterobacter cancerogenus* strain LMG 2693 (Genbank accession number 343206385). It is interestingly to note, that *Enterobacter* strain with antimicrobial activities was isolated from entry chiffon. Since the bacteria were isolated from the same environmental niche, the hypothesis was tested whether specific interaction may be observed among the isolates. When the isolates were tested against each other, a general pattern became apparent where the Gram-positive strains were active against Gram-positive bacteria and Gram-negative strains were active against Gram-negative. This strain also to produced cytotoxic activities targeted against eukaryotic host cell as this would harm the animal when produced under environmental conditions.

G. Fermentation for *Enterobacter cancerogenus* strain LMG 2693.

Time course of the growth of *Enterobacter cancerogenus* strain LMG 2693 in the agitation speed up to 120 rpm, temperature 30°C, pH 7, and glucose at concentration of 0,1 % w/v. The antimicrobial biosynthesis was strongly affected by different incubation temperatures its like 30°C and glucose affected the antimicrobial substance production. This result was in accordance with Hosny et al (2011) who pointed out that many fermentation require glucose as the carbon source, and that production may be controlled by catabolite repression

III. REFERENCES

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