

Ecology, Environment and Conservation Editorial Advisory Board

Chief Editor

Prof.(Dr.) R.K.Trivedy, Pune, India

EDITORIAL ADVISORY BOARD

- | | |
|--|--|
| 1. Dr. Teresa Ferreira, Portugal | 19. Dr. A. Olawale, Nigeria |
| 2. Dr. Michael Ukwuru, Nigeria | 20. Dr. Ing. Agr. Mario Ridardo Sabbatini, Argentina |
| 3. Dr. Moses Inbaraj, Chennai | 21. Dr. Philip C. Reid, U.K. |
| 4. Dr. D.J. Lee, Taiwan | 22. Dr. Mohd. Yusuf, Malaysia |
| 5. Dr. Christial Paul P.delacruz, Phillipnes | 23. Dr. Oswaldo A. Feernandez, Argentina |
| 6. Dr. T. Bahorun, Mauritius | 24. Dr. Ms. Mirela Tulik, Warsaw, Poland |
| 7. Dr. Linda Blackwell, Australia | 25. Dr. L.L. Chukwu, Nigeria |
| 8. Dr. G. Zellner, Netherlands | 26. Dr. Azni Idris, UPM, Malaysia |
| 9. Dr. Wilson S. Tisera, Kupang, Indonesia | 27. Dr. Vikas Sharma, J&K, India |
| 10. Dr. M.F. Hamoda, Kuwait | 28. Dr. Amresh Chandra Pandey, Jharkhand, India |
| 11. Dr. H.A.Abrahamse, South Africa | 29. Prof.(Dr.) Agoes Soegianto, Indonesia |
| 12. Dr. Arulmozhiyal R., Salem | 30. Dr. A.K. Panigrahi, Berhampur, India |
| 13. Dr. Hassan Ibrahim Ali, Sudan | 31. Dr. Ahmed El Mahmoudi, Saudi Arabia |
| 14. Dr. A.R.Ghosh, Burdwan, India | 32. Dr. Seyed Mohammad Tajbakhsh, Iran |
| 15. Prof. M. Zaman, Bangladesh | 33. Dr. Amin L. Setyo, Indonesia |
| 16. Dr. Marcantonio Bragdin, Venice, Italy | 34. Dr. Francis Gbogbo, Ghana |
| 17. Dr. Z. Fuat Topark, Turkey | 35. Dr. S. Shabanlou, Iran |
| 18. Dr. Z. Li. Bonn, Germany | |

[Back to EEC Journal Details](#)



Ecology, Environment and Conservation Journal Papers

Issue: Vol 25, Sept Suppl. Issue, 2019

[CELL WALL RESPONSE OF BACTERIA SERRATIAMARCESCENS LII61 – LIPASE AND PROTEASE ENZYMES PRODUCER – IN GRAM STAINING](#)

Fatimah, Rizka Diah Fitri, Gilvallavi, Elga Renjana, Intan Ayu Pratiwi, Ni'matuzahroh and Sri Sumarsih

[Get Abstract](#)

[THE CHARACTERIZATION OF POTENTIAL ADSORBENTS MADE OF SOLID TOFU WASTE IMMOBILIZED ON SILICA SURFACE FOR REMOVAL OF HEAVY METAL IONS](#)

Handoko Darmokoesoemo¹, Eko Prasetyo Kuncoro, Ganden Supriyanto and Yosephine Sri Wulan Manuhara

[Get Abstract](#)

[THE TRADITIONAL KNOWLEDGE OF TIMOR ETHNIC IN EAST BAUMATA IN UTILIZING PLANTS](#)

Andam S. Ardan

[Get Abstract](#)

[EFFECTIVITY OF COCONUT SHELL CHARCOAL AND MORINGAOLEIFERA POWDER IN DOMESTIC WASTE WATER TREATMENT](#)

Nur Aini Bunyani and Willem Amu Blegur

[Get Abstract](#)

[EVALUATION OF SOME FACTORS AFFECTING YIELD AND QUALITY OF VIRGIN COCONUT OIL \(VCO\) PRODUCED BY FERMENTATION USING BAKER YEAST](#)

Jasman*, Roswita M. P. Gabur, Nofriany M. Lede, Christine N. Lota, Resti A. Nubatonis, Sudirman and YosepLawa

[Get Abstract](#)

[ISLAMIC BOARDING SCHOOL'S STRATEGIES AND METHODS OF ENVIRONMENTAL CONSERVATION EDUCATION THROUGH THE APPLICATION OF ISLAMIC VALUES](#)

M. Harja Efendi, Mimien Henie Irawati, Fatchur Rochman and Abdul Gofur

[Get Abstract](#)

[RANCIDITY OF TRADITIONAL COCONUT OIL: MICROBIAL CONTAMINANT AND FREE FATTY ACID CONTENT](#)

Nur R. Adawiyah Mahmud*, Ernawati and Asmiati

[Get Abstract](#)

Search Articles

Journal Issues

[Vol 26, Issue 1, 2020](#)
[Vol 26, Feb Suppl. Issue, 2020](#)
[Vol 25, Issue 4 2019](#)
[Vol 25, Issue 3 2019](#)
[Vol 25, Nov Suppl. Issue, 2019](#)
[Vol 25, Sept Suppl. Issue, 2019](#)
[Vol 25, Aug Suppl. Issue, 2019](#)
[Vol 25, July Suppl. Issue, 2019](#)
[Vol 25, Issue 2 2019](#)
[Vol 25, May Suppl. Issue, 2019](#)
[Vol 25, April Suppl. Issue, 2019](#)
[Vol 25, Issue 1 2019](#)
[Vol 24, Issue 4 2018](#)
[Vol 24, Issue 3 2018](#)
[Vol 24, Issue 2 2018](#)
[Vol 24, Issue 1 2018](#)
[Vol 24, March Suppl. Issue 2018](#)
[Vol 24, Feb. Suppl. Issue 2018](#)
[Vol 23, Issue 4, 2017](#)
[Vol 23, Nov. Suppl. Issue 2017](#)
[Vol 23, Sept. Suppl. Issue 2017](#)
[Vol 23, Issue 2, 2017](#)
[Vol 23, Issue 3, 2017](#)
[Vol 23, Issue 1, 2017](#)
[Vol 23, Feb 2017 Suppl. Issue](#)
[Vol 22, Dec 2016 Suppl. Issue](#)
[Vol 22, Issue 4, 2016](#)
[Vol 22, Sept. Suppl. Issue, 2016](#)
[Vol 22, Issue 3, 2016](#)
[Vol 22, June Suppl. Issue 2016](#)
[Vol 22, Issue 2, 2016](#)
[Vol 22, April Suppl. Issue 2016](#)
[Vol 22, Issue 1, 2016](#)
[Vol 21, Issue 4, 2015](#)
[Vol 21 Dec. 2015 Suppl. Issue](#)
[Vol 21 Nov. 2015 Suppl. Issue](#)
[Vol 21, Issue 3, 2015](#)
[Vol 21, Issue 2, 2015](#)
[Vol 21 Suppl. Issue August 2015](#)
[Vol 21, Suppl. Issue June 2015](#)
[Vol 21, Issue 1, 2015](#)
[Supplement Issue, Dec. 2014](#)
[Special Issue-2014](#)
[Vol 20, Issue 4, 2014](#)
[Vol 20, Issue 3, 2014](#)
[Vol 20, Issue 2, 2014](#)

THE EFFECT OF EXTENSION STRATEGY AND SOCIAL STATUS ON FARMERS' KNOWLEDGE ABOUT LAND CONSERVATION (AN EXPERIMENT AT KECAMATAN LEMAH ABANG, KARAWANG)

Sobandi, Amos Neolaka and Yufiarti

[Get Abstract](#)

DETECTION OF TETRACYCLINE (TETA) GENE AND SULFONAMIDES (SULL) GENE IN ESCHERICHIA COLI ISOLATED FROM FRESH MILK IN SURABAYA, INDONESIA, USING POLYMERASE CHAIN REACTION TECHNIQUE

TridiganitaIntan Solikhah, Dentira Dewi Yusvarianty, Gahastanira Permata Solikhah, Maya Nurwartanti Yunita and Hartanto Mulyo Raharjo

[Get Abstract](#)

THE EFFECT OF VIRGIN COCONUT OIL WASTE IN THE RATION AGAINST BROILER CHICKEN GROWTH (GALLUS SP.)

Tridiganita Intan Solikhah and Gahastanira Permata Solikhah

[Get Abstract](#)

ENHANCEMENT OF FLAVONOID PRODUCTION OF GYNURAPROCUMBENS (LOUR.)MERR ADVENTITIOUS ROOTS IN BALOON TYPE BUBBLE BIOREACTOR INFLUENCED BY PHENYLALANINE AND TYROSINE

Yosephine Sri Wulan Manuhara, Rine Noviyanti, Popy Hartatie Hardjo and MulyadiTanjung

[Get Abstract](#)

PHYTOCHEMICAL SCREENING AND TOTAL PHENOL CONTENT OF PACAR AIR (IMPATIENS BALSAMINA L) LEAVES EXTRACT

Rizka Diah Fitri, Gilvallavi, Nadiah Al Batati, Muhammad Sholeh Al Qoyyim, Lilik Hidayatul Mukminin2, Betty Lukiati, Sri Sumarsih and Fatimah

[Get Abstract](#)

ANALYSIS OF STUDENT UNDERSTANDING OF BIOLOGICAL LEARNING MODEL USING CURRENT MUSHROOM MEDIUM (PLEUROPTUSOSTREATUS) AS SOGER MEAT

Elisje Theodora Maasawet, Evie Palenewen and Wahyu Sekti Retnaningsih

[Get Abstract](#)

THE EFFECT OF ETHANOL EXTRACTS OF RED POMEGRANATE PEEL(PUNICAGRANATUM L.) ON ACTIVITIES CANDIDA ALBICANS

Lolita A.M. Parera, YosepLawa and Evansia More

[Get Abstract](#)

SOIL ANALYSIS AS AN INDICATOR OF SOIL FERTILITY CONTAMINATED WITH PLASTIC WASTE IN THE CITY OF KEFAMENANU

Hermina Manlea and Yohana Ivana Kedang

[Get Abstract](#)

EFFECT OF EDUCATIONAL FACTORS ON THE USE OF CLEAN WATER IN RURAL COMMUNITIES IN THE DRY TROPICAL AREAS, EAST NUSA TENGGARA – INDONESIA

Jakobis Johanis Messakh and Herles C. Zakarias

[Get Abstract](#)

PREPARATION OF HYDROXYAPATITE BASED ON NANO CORAL USING PRECIPITATION METHOD: INFLUENCE OF MOLARITY OF PHOSPHORIC ACID AND SINTERING

[Vol. 20 Issue 01, 2014](#)

[Vol. 19 Issue 04, 2013](#)

[Vol. 19 Issue 03, 2013](#)

[Vol. 19, Issue 02, 2013](#)

[Vol. 19, Issue 01, 2013](#)

[Vol.18, Issue 04, 2012](#)

[Vol.18, Issue 3, 2012](#)

[Vol.18, Issue 2, 2012](#)

[Vol.18, Issue 1, 2012](#)

[Vol.17, Issue 4, 2011](#)

[Vol.17, Issue 3, 2011](#)

[Vol.17, Issue 2, 2011](#)

[Vol.17, Issue 1, 2011](#)

[Vol.16, Issue 4, 2010](#)

[Vol.16, Issue 3, 2010](#)

[Vol.16, Issue 2, 2010](#)

[Vol.16, Issue 1, 2010](#)

[Vol.15, Issue 04, 2009](#)

[Vol.15, Issue 03, 2009](#)

[Vol.15, Issue 02, 2009](#)

[Vol.15, Issue 1, 2009](#)

[Vol.14, Issue 04, 2008](#)

[Vol.14, Issue 2-3, 2008](#)

[Vol.14, Issue 2-3, 2008](#)

[Vol.14, Issue 1, 2008](#)

[Vol.14, Issue 2-3, 2008](#)

[Vol.13, Issue 04, 2007](#)

[Vol.13, Issue 2, 2007](#)

[Vol.13, Issue 1, 2007](#)

[Vol.12, Issue 4, 2006](#)

[Vol.12, Issue 3, 2006](#)

[Vol.12, Issue 2, 2006](#)

[Vol.12, Issue 1, 2006](#)

[Vol.12, Issue 01, 2006](#)

[Vol.11, Issue 3,4, 2005](#)

[Vol.11, Issue 2, 2005](#)

[Vol.11, Issue 1, 2005](#)

[Vol.10, Issue 04, 2004](#)

[Vol.10, Issue 03, 2004](#)

[Vol.10, Issue 02, 2004](#)

[Vol.10, Issue 01, 2004](#)

[Vol.09, Issue 04, 2003](#)

[Vol.09, Issue 03, 2003](#)

[Vol.09, Issue 02, 2003](#)

[Vol.08, Issue 04, 2002](#)

[Vol.08, Issue 03, 2002](#)

[Vol.08, Issue 01, 2002](#)

[Vol.07, Issue 04, 2001](#)

[Vol.07, Issue 03, 2001](#)

[Vol.07, Issue 02, 2001](#)

[Vol.07, Issue 01, 2001](#)

[Vol.06, Issue 04, 2000](#)

[Vol.06, Issue 03, 2000](#)

[Vol.06, Issue 02, 2000](#)

[Vol.06, Issue 01, 2000](#)

[Vol.05, Issue 04, 1999](#)

[Vol.05, Issue 03, 1999](#)

[Vol.05, Issue 02, 1999](#)

[Vol.05, Issue 01, 1999](#)

[Vol.04, Issue 1,2, 1998](#)

[Vol.03, Issue 3,4, 1997](#)

[Vol.03, Issue 01, 1997](#)

[Vol.02, Issue 1,2, 1996](#)

[Vol.01, Issue 14, 1995](#)

TEMPERATURE

Siswanto, Dyah Hikmawati, Wahyuning Tyastutik and Desy Herlinawati

[Get Abstract](#)

Looking for Past Issues?
[Click here to get them!!](#)

TRANSITION FROM RED LIGHT DISTRICT TO SMALL BUSINESS DISTRICT FOR SUSTAINABLE URBAN

D.S. Dewi, Setiawan, M. Yusuf, Waluyohadi and N.I. Sari

[Get Abstract](#)

SUSTAINABILITY OF KALIBOKOR CANAL DUE TO COMMUNITY LIVELIHOOD ALONG THE BANKS

Eddy Soedjono, Y.H. Ridwan, Beiby Tangahu and K.P. Tucunan

[Get Abstract](#)

THE GEMEINSCHAFT CITY: PARADIGMS AND MEASUREMENTS OF SOCIAL SYSTEM IN URBAN AREA

K.P. Tucunan, Y.H. Ridwan1, Sutikno, Setiawan and E.S. Soedjono

[Get Abstract](#)

PRODUCTION OF BIO-BRIQUETTE FROM BIOCHAR DERIVED FROM PYROLYSIS OF CASHEW NUT WASTE

La Ifa, Setyawati Yania, Nurjannah Nurjannah, Zakir Sabara, Yuliana Yuliana, Heri Septya Kusuma and Mahfud Mahfud

[Get Abstract](#)

[Home](#) | [International Journals](#) | [Books](#) | [Environmental Consulting](#) | [About Us](#) | [Contact Us](#) | [Submit Paper](#) | [Search Journal Article](#) |

[Become a fan](#) on Facebook

[Follow us](#) on Twitter



© EM International 2012-2019 | **Developed by Eneblur Consulting**

molecutrack

We Focus on Clean Energy Solutions That Replace Hazardous and Carbon Intensive Fuel.

molecutrack.com OPEN

Ecology, Environment and Conservation

12

H Index

Country
Subject Area and Category

India -  [SJR Ranking of India](#)

[Agricultural and Biological Sciences](#)
[Ecology, Evolution, Behavior and Systematics](#)

[Environmental Science](#)
[Ecology](#)
[Nature and Landscape Conservation](#)

Publisher

[EM International](#)

Publication type

Journals

ISSN

0971765X


Coverage

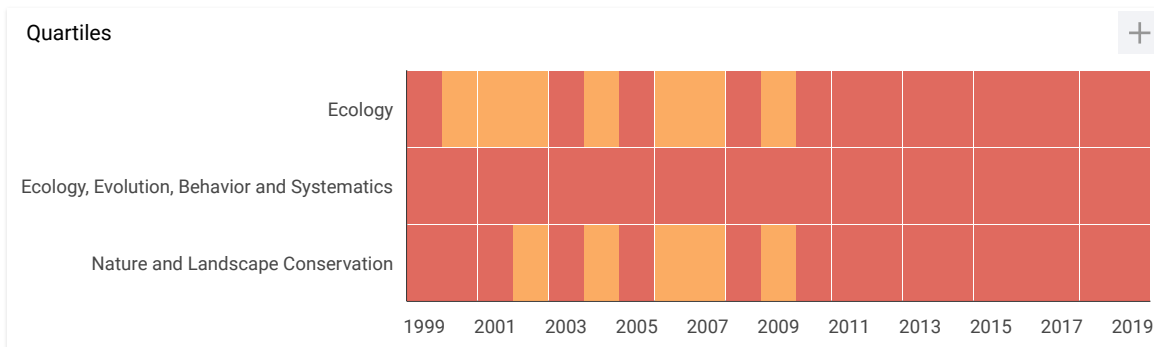
1997-2019

Scope

Published Quarterly Since 1995. Ecology, Environment and Conservation is published in March, June, September and December every year. ECOLOGY, ENVIRONMENT AND CONSERVATION is one of the leading international environmental journal. It is widely subscribed in India and abroad by Institutions and Individuals in education and research as well as by Industries, Govt. Departments and Research Institutes.



- [Homepage](#)
- [How to publish in this journal](#)
- [Contact](#)
-  [Join the conversation about this journal](#)

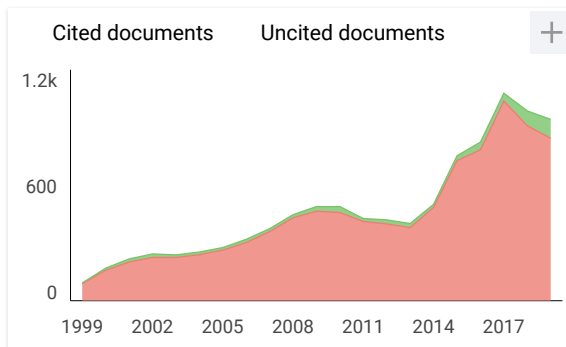
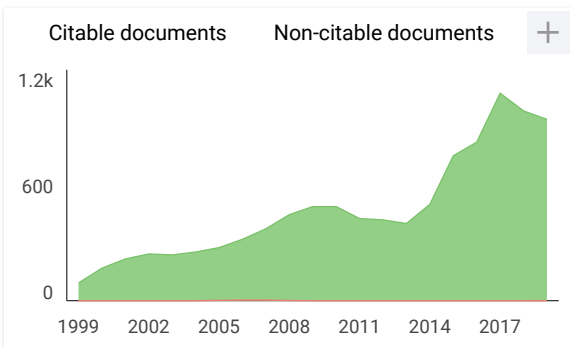
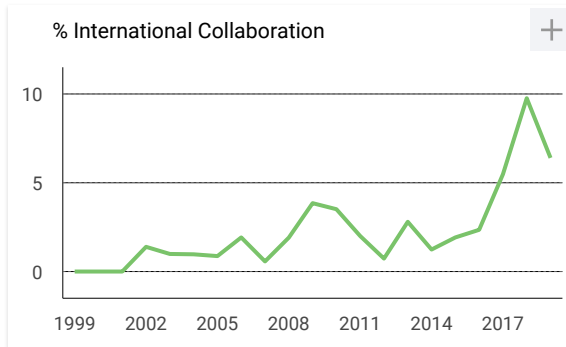
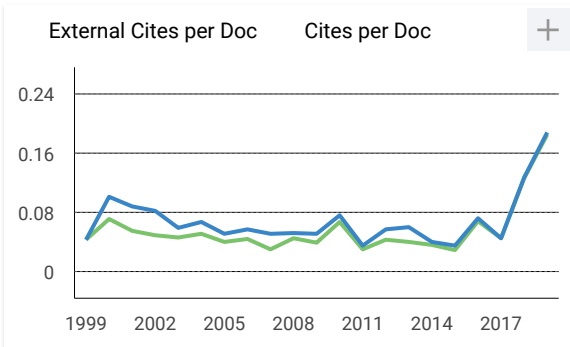
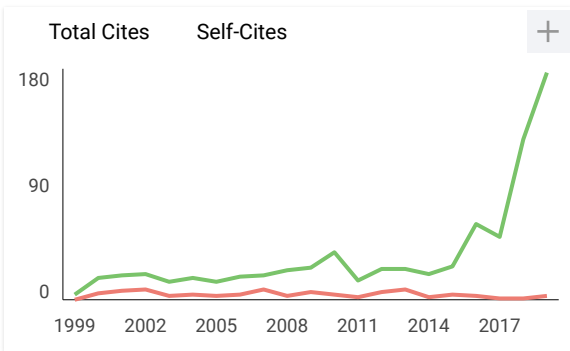
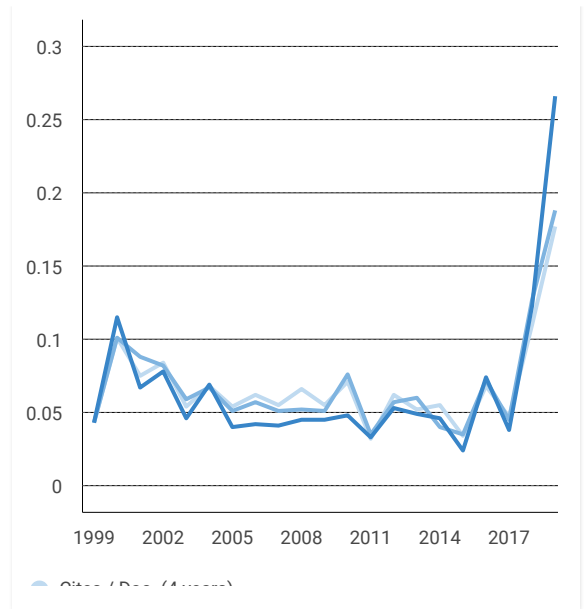
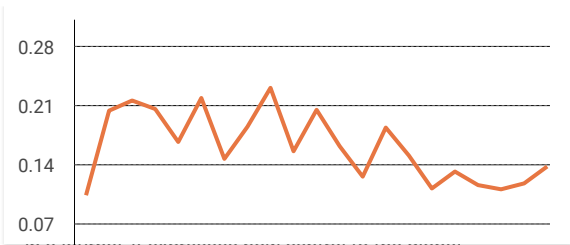


SJR



Citations per document





Ecology, Environment and Conservation

Q4 Ecology
best quartile

SJR 2019
0.14

powered by scimagojr.com

← Show this widget in your own website

Just copy the code below and paste within your html code:

```
<a href="https://www.scimaç
```

L **Leta Rafael Levis** 2 weeks ago

I have published my article on this journal in 2017, no 23 Issue 2 with the title of the article was :
Farmers attitude
How ever, I have got the URL for full text.
So, please let me know the URL for the article full text.

Change my email address: in 2017 when I sent my article to this journal I still use email address:

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/336304893>

Cell wall response of bacteria *Serratia marcescens* LII61 –lipase and protease enzymes producer –in gram staining

Article in *Ecology, Environment and Conservation* · September 2019

CITATION

1

READS

223

7 authors, including:



Fatimah Fatimah

Airlangga University

20 PUBLICATIONS 15 CITATIONS

SEE PROFILE



Elga Renjana

Indonesian Institute of Sciences

5 PUBLICATIONS 2 CITATIONS

SEE PROFILE



Intan Ayu Pratiwi

Airlangga University

4 PUBLICATIONS 1 CITATION

SEE PROFILE



Ni'matuzahroh Ni'matuzahroh

Airlangga University

52 PUBLICATIONS 143 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Fungi biodiversity [View project](#)



Apprenticeship [View project](#)

Cell wall response of bacteria *Serratia marcescens* LII61 – lipase and protease enzymes producer – in gram staining

Fatimah^{*1,3}, Rizka Diah Fitri¹, Gilva Illavi¹, Elga Renjana¹, Intan Ayu Pratiwi¹, Ni'matuzahroh^{1,3} and Sri Sumarsih²

¹Department of Biology, Faculty of Science and Technology, Airlangga University, 60115 Surabaya, Indonesia

²Department of Chemistry, Faculty of Science and Technology, Airlangga University, 60115 Surabaya, Indonesia

³Research Center for Bio-Molecule Engineering, Airlangga University, 60115, Surabaya, Indonesia

(Received 24 May, 2019; Accepted 28 July, 2019)

ABSTRACT

This study was aimed to reveal cell wall response of *Serratia marcescens* LII61 to Gram staining and its lipase and protease activities. Microscopic characters were obtained through the Gram staining procedure performed on variations in the age of bacterial cultures (16, 18, 24, 48, and 72 hours) which were grown on Luria Bertani Broth medium. Lipase activity assay was carried out using a spectrophotometer, with lipolytic selective media and observed every 4 hours for 24 hours. Protease activity was carried out using spotted method on proteolytic selective media and observed at 48 hours incubation time. The results of this study on Gram staining showed that *S. marcescens* LII61 has a Gram variable character, which was indicated by the change in color of the cell wall resulting from gram staining at the observed age of the culture. The highest lipase activity was produced at 16 hours after incubation (29.39 U/mL) and protease activity was indicated by hydrolysis index at 48 hours i.e 1.2. The cell wall response of the *S. marcescens* LII61 is an interesting matter to discuss because so far the bacterium *Serratia marcescens* is known as bacil Gram negative. The presence of gram variable properties shown in the results of this study can be attributed to the ability of bacterial cell wall to adapt in unsuitable nutrient condition, pH, temperature and electrolyte content in the environment.

Keywords: Culture age, Gram variable, *Serratia marcescens* LII61

Introduction

Indonesia is rich in biodiversity of microbes and biomass. Based on its geographical condition, Indonesian indigenous microbes have capability to produce superior enzyme, including lipase and protease, or better known as lipolytic and proteolytic microbes. At present, the use of lipase and protease enzymes in the food and non-food industries is in-

creasing. In the food industry, lipase is widely used in the dairy industry, bread and cake industry, beer industry, spice industry, and processing meat and fish. Therefore, lipase is also used in the non-food industry, such as chemical and pharmaceutical industries, oleochemical industry, detergent industry, pharmaceutical industry, medicine, cosmetics industry and waste treatment industry (Momsia and Momsia, 2013; Farrokh *et al.*, 2014; Guerand, 2017).

Corresponding author's email: fatimah@fst.unair.ac.id

Meanwhile, protease play roles in protein breakdown reactions. The use of protease in industrial fields include the industries of detergent, leather, textile, food, protein hydrolysate, milk processing, pharmaceutical, beer, and waste processing industry (Moon and Parulekar, 1993).

Fatimah *et al.*, (2011) has succeeded in isolating bacteria producing lipase and protease from slaughterhouse waste in North Surabaya. One of the isolate is LII61 which were also investigated for their ability to degrade oil sludge (Ni'matuzahroh *et al.*, 2017), and produce bio surfactants (Renjana *et al.*, 2017).

Ni'matuzahroh *et al.*, (2017) has revealed the macroscopic character of colony, cell microscopy, and physiology of LII61 isolate. The study showed that LII61 isolate were identified as *Micrococcus* sp. LII61 uses a microbact 12A/12B identification kit. Microscopic character of LII61 isolate showed Gram-positive cocci (Renjana *et al.*, 2017) identified using 16S rRNA molecular marker; the result showed that LII61 isolate were identified as *S. marcescens* with 99% similarity degrees. So far, *S. marcescens* is classified in Gram-negative bacteria. Based on this background, this study aims to confirm the status of species from LII61 isolate molecularly, observe the response of this bacterial cell wall to Gram staining at different age cultures, and assay the activity of lipase and protease hydrolysis index.

Materials and Methods

Macroscopic Characterization

S. marcescens LII61 bacterial colonies from stock culture were stricked on Nutrient Agar medium using the quadrant method. Colony characterization was carried out on single colonies 24 hours culture age, which included shape, diameter, color, margin, colony texture, and elevation of the colonies.

Microscopic Characterization

Observation of cell microscopic characterization included cell shape and cell wall response to gram staining using HUCKER staining kit. Culture of *S. marcescens* LII61 bacteria was first cultured in Luria Bertani Broth medium and gram staining was carried out at 16, 18, 24, 48 and 72 hours of culture.

Lipase Activity Assay

The medium for lipolytic assay (100 mL) consist of

0.2 g yeast extract, 0.05 g $MgSO_4 \cdot 3H_2O$, 0.23 g NaCl, 0.5 g K_2HPO_4 , 1% cooking oil, and distilled water. Determination of lipase enzyme activity was carried out by the method of Pereira *et al.*, (1997) in modified Sumarsih, (2005). Lipase enzyme activity was determined by spectrophotometry using a *p*-nitro phenyl palmitate substrate (*p*-NPP). Lipase activity of *S. marcescens* LII61 was measured every 4 hours for 24 hours of incubation.

Protease Activity Assay

The medium for proteolytic assay with a mixture of Bussnell Hass Agar containing skim milk. The bacterium was inoculated as small spot into the medium and incubated at 37 °C. The appearance of clear zones around the colonies after 48 hours of incubation show proteolytic activity. Measurement of protease activity was carried out according to the method performed by Melliawati *et al.*, (2015).

Molecular Identification of *Serratia marcescens* LII61

Isolation of genomic DNA from *S. marcescens* LII61 using the Wizard® DNA Purification Kit (Promega). Measurement of purity and DNA concentration was carried out using Thermo Scientific TM Multiskan GO Microdroplet Spectrophotometer at wavelengths of 260 nm and 280 nm. The gene amplification procedure is adjusted to the direction of the PCR Master Mix Protocol (Promega) manufacturer. Molecular marker of the 16S rRNA gene determined bacterial identification, primers used to amplify the 16S rRNA gene were universal primers, namely: P0F-5'-GAG AGT TTG ATC CTG GAG CAG-3 'and P6R-5'- CTA CGG CTA CCT TGT TAC GA-3 ' (Chakraborty *et al.*, 2015).

The PCR conditions used were pre-denaturation temperature of 95 °C for 1 minute, denaturation temperature of 95 °C for 30 seconds, annealing 55 °C for 45 seconds, elongation of 72 °C for 1 minute, and final extension 72 °C for 5 minutes. Confirmation of genomic DNA and PCR product was carried out using agarose gel electrophoresis with a concentration of 1%, at 135 volts for 25 minutes. Electrophoresis results were visualized at UV-Transluminator then documented with a geldoc camera.

Amplicon DNA was sequenced at First Base Laboratories using the Bigdye® Terminator v3.1 Cycle Sequencing kit and analyzed using the ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, USA). The DNA sequencing results were edited using the

BioEdit Sequence Alignment Editor version 7.2.5 software. Then analyzed using the BLAST program in NCBI (<https://www.ncbi.nlm.nih.gov/>).

Results and Discussion

Macroscopic Characteristics of Colonies

S. marcescens LII61 bacterial colonies on Nutrient Agar media with a 24 hours culture age can be found in Figure 1 and colony characters showed in Table 1.



Fig. 1. Colonies of *Serratia marcescens* LII61 bacteria on Nutrient Agar media at 24 hours culture age

Table 1. Characteristics of *Serratia marcescens* LII61 colonies

No	Colony Character	Colony Character <i>Serratia marcescens</i> LII61
1	Colony Diameter	3.3 mm
2	Colony colors	Red
3	Margin	Entire
4	Colony texture	Mucoid
5	Elevation	Umbonate

S. marcescens LII61 has red colonies that are not always found in some other *Serratia* species (Barrow

and Feltham, 2003). It can produce specific secondary metabolites that is prodigiosin (Elkenawy *et al.*, 2017).

Microscopic Characteristics

The cell of *S. marcescens* bacteria LII61 was a short rod at 16 and 18 hours and change into cocci at a culture age above 24 hours. The results gram staining for *S. marcescens* LII61 at 16, 18, 24, 48, and 72 hours can be found in Figure 2.

Based on the result changing in response to gram staining of *S. marcescens* LII61 bacteria can be categorized into gram variable, gram-negative properties were shown at 16 to 18 hours of age culture, and begin to show gram positive at the age above 24 hours. Smolin *et al.*, (2005) also express the gram variable nature of this bacterial species.

In various literature, it is mostly explained that *S. marcescens* include gram negative bacteria (Elkenawy *et al.*, 2017; Lee *et al.*, 2007; Li *et al.*, 1995; Panizza *et al.*, 2013), but in this study indicates that this species belongs to the gram variable. The difference in the result of gram staining can be influenced by various environmental conditions, which cause the structure of the cell wall to change. According to Beveridge, (1990), in certain eubacterial groups; the response of gram staining is erratic caused by unsuitable nutrient, temperature, pH or other electrolyte content. However, in specific bacterial, the nature of gram variable will be different in under optimal condition. Based on description, the result of gram staining in *S. marcescens* LII61 species are caused by change in the formation of the peptidoglycan layer on the cell wall structure. This is influenced by the environmental conditions of the bacteria. This bacteria species is found in slaughterhouse wastes, which contains nutrition, pH and electrolytes can affect the mechanism of peptidoglycan layer formation in the structure of cell wall.

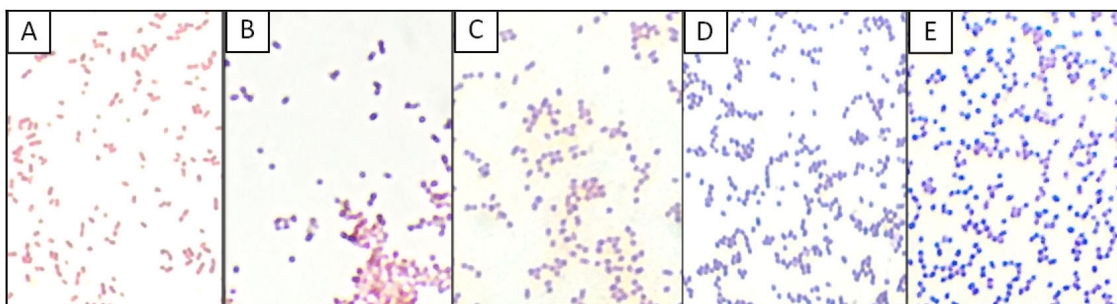


Fig. 2. Gram staining of *Serratia marcescens* LII61. (A, B, C, D, E: 16, 18, 24, 48, 72 hours M 1000x)

Lipase Activity Assay

Lipase activity of *S. marcescens* LII61 bacteria from each time of observation during 24 hours incubation is shown in Fig. 3.

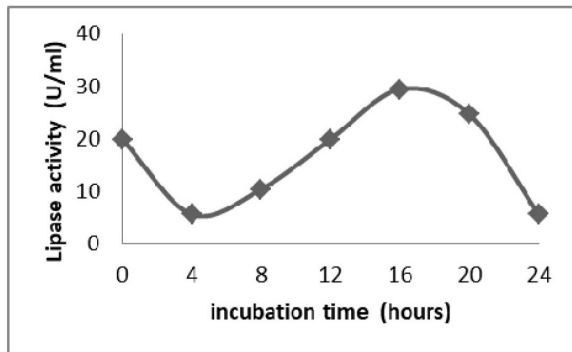


Fig. 3. Lipase activity of *S. marcescens* LII61 bacteria at different incubation times

The highest activity was registered at 16 hours (29.39 U/mL). The activity of lipase enzyme can be related to the phase of bacterial growth. Maximum lipase activity was obtained at 16 hours of incubation. This result is appropriate with Gururaj *et al.*, (2016) which works with *Acinetobacter* sp. and obtain maximum lipase activity at 16 hour of incubation. Based on this result, *S. marcescens* LII61 shows important characteristic for industries because this strain will be preferred if it has higher activity and shorter incubation time (Niyonzima and More, 2014).

Protease Activity Assay

The results showed that *S. marcescens* LII61 can produce protease enzyme. The clear zone formed

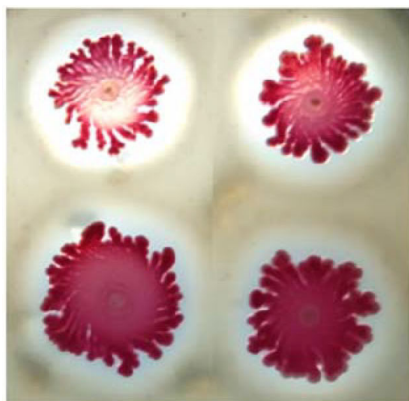


Fig. 4. Proteolytic activities of *Serratia marcescens* LII61 at 48 hours.

around the colonies is caused by the casein substrate in the media has been hydrolyzed into peptide and amino acid compounds (Nurkhasanah and Widodo, 2015). Casein as a substrate will induce protease enzyme activity and bacteria that hydrolyze casein are characterized by the presence of clear zones around the colonies (Atlas, 2010). The results of the protease activities assay were shown in Figure 4.

The ability of protease activity can be seen based on the proteolytic index. Observations on incubation 48 hours showed that the average proteolytic index is 1.20.

Molecular Identification of *Serratia marcescens* LII61

The purity and concentration of DNA is categorized as good because it has a purity value between 1.8-2.0, which is 1.93 and a concentration of 35.1 ng/ μ L. Amplification of the 16S rRNA gene from *S. marcescens* LII61 using the PCR method visualized in Fig. 5. The DNA band obtained had a size of \pm 1300 bp.

Based on the confirmation results of the 16S rRNA gene by electrophoresis, a single band was obtained, and then it was followed by sequencing at the 1st Base Sequencing Service, Singapore. Sequencing data were analyzed using the BioEdit Sequence Alignment Editor version 7.2.5. The sequence of *S. marcescens* LII61 16S rRNA gene has a size of 1045 base pairs. Based on BLAST analysis, the 16S rRNA gene from *S. marcescens* LII61 has similarity (98.56%)

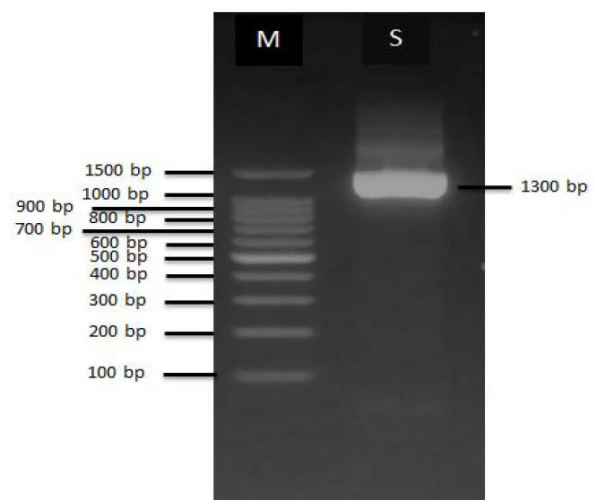


Fig. 5. 16S rRNA gene amplification of *Serratia marcescens* LII61 bacteria in 2% agarose gel. M: DNA Marker 100 bp; S: sample.

with *S. marcescens* strain ADY09 (acc. number MH084798.1). So the results can be concluded that the species used in this study is *S. marcescens* strain LII61 has a red colony character and gram variable.

Conclusion

Based on this research, *S. marcescens* LII61 is gram variable, has red colony and similarity (98.56%) with *S. marcescens* ADY09. This bacterium can produce lipase (29.39 U/mL) at 24 hours after incubation and protease with hydrolytic index is 1.2 at 48 hours.

References

- Atlas, R. M. 2010. *Handbook of Microbiological Media Fourth Edition*. Washington D. C: ASM Press.
- Barrow, G. I. and Feltham, R. K. A. 2003. *Cowan and Steel's Manual for The Identification of Medical Bacteria*. Cambridge University Press, Australia.
- Beveridge, T. J. 1990. Mechanism of Gram Variability in Select Bacteria. *Journal of Bacteriology*. 172(3) : 1609–1620.
- Chakraborty, S., Ghosh, M., Chakraborti, S., Jana, S., Sen, K. K., Kokare, C. and Zhang, L. 2015. Biosurfactant Produced from *Actinomyces nocardiformis* A17: Characterization and its Biological Evaluation. *International Journal of Biological Macromolecules*. 79 : 405–412.
- Elkenawy, N. M., Yassin, A. S., Elhifnawy, H. N. and Amin, M. A. 2017. Optimization of Prodigiosin Production by *Serratia marcescens* Using Crude Glycerol and Enhancing Production Using Gamma Radiation. *Biotechnology Reports*. 14 : 47–53.
- Farrokh, P., Yakhchali, B. and Karkhane, A. A. 2014. Cloning and Characterization of Newly Isolated Lipase from *Enterobacter* sp. Bn12. *Brazilian Journal of Microbiology*. 45 (2) : 677-687.
- Fatimah, Ni'matuzahroh, and Nurhariyati, T. 2011. *Eksplorasi Bakteri Proteolitik dan Lipolitik dari Limbah Rumah Potong Hewan, Surabaya*. Surabaya.
- Guerrand, D. 2017. Lipase Industrial Application: Focus on Food and Agroindustries. *Oilseeds and Fats Crops and Lipids*. 24(4).
- Gururaj, P., Ramalingam, S., Devi, G. N. and Gautam, P. 2016. Process Optimization for Production and Purification of a Thermostable, Organic Solvent Tolerant Lipase from *Acinetobacter* sp. AU07. *Brazilian Journal of Microbiology*. 47(3) : 647–657.
- Lee, K. W., Bae, H. A. and Lee, Y. H. 2007. Molecular Cloning and Functional Expression of *esf* Gene Encoding Enantioselective Lipase from *Serratia marcescens* ES-2 for Kinetic Resolution of Optically Active (S)-flurbiprofen. *Journal of Microbiology and Biotechnology*. 17 (1) : 74–80.
- Li, X., Tetling, S., Winkler, U. K., Jaeger, K. E. and Benedik, M. J. 1995. Gene Cloning, Sequence Analysis, Purification, and Secretion by *Escherichia coli* of an Extracellular Lipase from *Serratia marcescens*. *Appl. Environ. Microbiol.* 61(7) : 2674–2680.
- Melliawati, R., Djohan, A.C. and Yopi. 2015. Selection of Lactic Acid Bacteria as a Producer of Protease Enzymes. *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia*. 1(2): 184-188.
- Momsia, T. and Momsia, P. 2013. A Review on Microbial Lipase Versatile Tool for Industrial Applications. *International Journal of Life Sciences Biotechnology and Pharma Research*. 2(4).
- Moon, S. and Parulekar, S. J. 1993. Some Observations on Protease Production in Continuous Suspension Cultures of *Bacillus firmus*. *Biotechnology and Bioengineering*. 41(1) : 43–54.
- Ni'matuzahroh, Pratiwi, I. A., Surtiningsih, T., Fatimah, and Sumarsih, S. 2017. The Potency of *Micrococcus* sp. LII61 Bacteria as Oil Sludge Cleaning Agent. *Journal of Biological Researches*. 22 (2): 38-42.
- Niyonzima, F. N. and More, S. S. 2014. Concomitant Production of Detergent Compatible Enzymes by *Bacillus flexus* XJU-1. *Brazilian Journal of Microbiology*: 45(3): 903–910.
- Nurkhasanah, S. and Widodo, 2015. The Effect of Different Media Content on Protease Activity *Bacillus subtilis*. *Jurnal Biotropika*. 3(2) : 104-106.
- Panizza, P., Syfantou, N., Pastor, F. I. J., Rodriguez, S. and Diaz, P. 2013. Acidic Lipase Lip I. 3 from a *Pseudomonas fluorescens* Like Strain Displays Unusual Properties and Shows Activity on Secondary Alcohols. *Journal of Applied Microbiology*. 114(3) : 722-732.
- Pereira-Meirelle, F. V, Rocha-Leão M.H.M. and Sant'Anna, J. G. L. 1997. A Stable Lipase from *Candida lipolytica*: Cultivation Condition and Crude Enzyme Characteristics. *Appl. Biochem. Biotechnol.* 63-65 : 73-85.
- Renjana, E., Helga, L., Fatimah, Sumarsih, S. and Ni'matuzahroh. 2017. Co-Utilization of Molasse and Glycerol as Carbon Sources on the Production of Biosurfactant by Isolate Bacteria LII61. *J. Applied Environmental and Biological Science*. 7(11) : 174-180.
- Smolin, G., Foster, C. S., Azar, D. T. and Dohlman, C. H. 2005. *Smolin and Thoft's the Cornea: Scientific Foundations and Clinical Practice*. Lippincott Williams & Wilkins.
- Sumarsih, S. 2005. *Skrining Bakteri Penghasil Lipase Termotabil dari Reaktor pada Pabrik Minyak Goreng. FMIPA*. Universitas Airlangga. Surabaya.