ISSN 2373-423X

JOAAT

Journal of Advanced Agricultural Technologies

Vol. 3, No. 3, September 2016

www.joaat.com

ISSN: 2373-423X (Print)

ISSN: 2301-3737(Online)

Journal of

Advanced Agricultural Technologies

Vol. 3, No. 3, September 2016

ISSN 2373-423X
JOAAT
Journal of Advanced Agricultural Technologies

Journal of Advanced Agricultural Technologies (JOAAT)

Journal of Advanced Agricultural Technologies

CONTENTS

Volume 3, Number 3, September 2016

A Simple Bacteriophage-Based Detection Kit for Detecting *Escherichia coli* on Post-Harvest Agricultural Product

Febrian E. S. Iriyanto[1,2], Wahyu C. Yuliasari[1], Arie Rahmawati[1], Muhammad Sholehuddin[1], Angga A. Ramadhan[1], and Hardian S. Addy[1,2]
[1]Faculty of Agriculture, University of Jember, Jember, East Java, Indonesia
[2]Center for Development of Advanced Sciences and Technology, University of Jember, Jember, East Java, Indonesia
Email: {febrianekashandy, wahyucici06, elfarie, tangankuusil, anggaadityar5}@gmail.com, hsaddy.faperta@unej.ac.id

***Abstract*—Contaminating food-borne pathogen such as *Escherichia coli* become one problem in food safety especially for agricultural product. Detection of the presence of pathogen may help customers to protect themselves against food-borne disease. Several simple techniques have been developing including the use of Total Plate Count (TPC). However, this method is time consumed resulting developing new approach in detecting *E. coli* including the use of specific bacteriophage. This research was aimed to isolate, formulate, and test the potency of bacteriophage in detecting *E. coli* which is isolated from post-harvest horticultural crops. About ten isolates were isolated from 5 traditional vegetable markets in Jember area. In addition, 2 kinds of bacteriophages were also isolated from vegetables and sewage for ϕPst-1 and ϕKR-1, respectively. Formulation of detection solution were based on Luria Bertani Modified Medium (LBMM) composted by LB + 1% Glucose (Glu) and 0.1% of Bromothymol Blue (BTB) which was selected based on stability of green-color at 570nm. Detection assay was also done using mixture of detection solution, sample, and bacteriophage ϕPsT-1 which was incubated at both room temperature and 37°C. The result showed that, the kit was able to determine the presence of *E. coli* about 4-5 hours depend on the incubation temperature. Negative result was shown by no color change while positive (contaminated by *E. coli*) result shown by the changing of color from green to yellow. Through this result we claimed that the technique is more efficient than TPC methods.**

***Index Terms*—*Escherichia coli*, food-borne disease, Detection, bacteriophage**

I. Introduction

Post-harvest agricultural product quality is the initial quality expression of groceries. Food safety on the food must be considered to avoid illness risk when peoples consume it [1]. Food safety recently well-considered by the government because of much contamination of food-borne pathogens. One of the contamination case is the presence of pathogenic bacterial on agricultural product such as milk, vegetables, and fruits. Contamination might be caused by microorganism which has an ability to infect peoples and cause a disease. Contamination might be caused by bacteria from contaminated meat which is able to contaminate nearby agricultural product such as vegetables, and fruits [1]. In some countries such as United States of America, 13% of microbe contamination caused by workers. Because of that, the cleanliness of food processing process must be well-considered [2].

One of the contaminant is food-borne bacterial pathogens *Escherichia coli* which is able to cause indigestion (*gastroenteritis)* [2]. Besides that, *E. coli* is also able to cause pneumonia, meningitis and abscess on human organs [3]. Antibiotic is commonly used to heal peoples. More than 90% of antibiotics produced by *Streptomyces* has been used on bacterial infections therapy. But those are mostly ineffective because of the high rates of bacterial antibiotics resistance [4].

Most of the post-harvesting agricultural product nowadays are not concerned well on bacterial contamination. *E. coli* presence detection usually done by using Total Plate Count (TPC) Methods which needs a long time process about 18-24 hours [5]. Besides that, on a research done by Ref. [6], *E. coli* presence detection can be done by singlepath *E. coli* O157 test more than 24 hours. Because of that, innovative and simple tools to quickly detect the contamination of *E. coli* is certainly needed. Bacteriophage known widely as a virus particle which is able to infect a specific bacteria [7]. Ref. [8] bacteriophage can be used to indicate the presence of pathogens quickly and specific.

II. Materials and Methods

A. Bacterial Isolation

E. coli isolate isolated from five different traditional markets and one vegetables dealer around Jember district area. The bacteria isolated from 10^{-5} diluted dishwater of horticulture product and the bacteria grew on Eosin-Methylene Blue Agar (EMBA) by pour plate methods by 50µl of dilution. Then the single colony of bacteria grew on Luria Bertani medium (LBM). The multiplication process done for 12 up to 24 hours at 37°C.

Manuscript received August 17, 2015; revised January 11, 2016.

doi: 10.18178/joaat.3.3.197-201

B. Bacteriophage Isolation

The bacteriophage isolated from dishwater of horticulture product and also soil around the vegetables dealer in Jember district area. 30ml dishwater and sewage also 100µl of bacterial culture mixed on 3ml LBM. The mixture incubated for 24 hours. The incubated mixture than filtered by 0,45µm membrane filter. The filtrate is the bacteriophage isolate and will be used to do spot test.

C. Bacteriophage Purification

The bacteriophage on the plaque of spot test propagated and purified from single plaque formed on *E. coli* culture. The 12 hour incubated bacteria in LBM and reach 0,1 optical density (OD_{600}) diluted 50 times by 50ml aquadest and mixed with 9ml LBM in 250ml Erlenmeyer flask. Then the bacteriophage inoculated to the mixture by *multiplicity of infection* (m.o.i.) 1.0. After incubated at 37°C for 24 hours, the bacterial cell separated from the medium by using centrifuge at 12.000 rpm for 15 minutes at 4°C. The supernatant then filtered by 0,45 µm membrane filter. The pure bacteriophage particles than saved at 4°C storage.

D. Production of E. coli Detection Kit

To know the ability of *E. coli* detection kit, a test done to find the range of detection time. 15µl inoculum suspension of *E. coli* (at 10^2, 10^3, 10^4, and 10^5 CFU/ml) poured into detection well (96-well microplate), then 100 µl detection liquid and 15µl bacteriophage poured into the same well. Modified EMB medium used as the control. The microplate then covered by aluminum foil and incubated at 37°C for 5 hours. Observation done by concerning at the color changes of the liquid in the well every 1 hour by using Hitachi U-2000 Spectrophotometer at 570nm wavelength.

E. Data Analysis

Data Analysis done by using ANOVA with 5% significance level continued by Least Significance Difference (LSD). The result than will be analysed and will be an Absorbance Graph (A). Data interpretation done by comparing the *E. coli* sample and the control sample treatment. The lower absorbance level compared to the control sample means the positive result of detection.

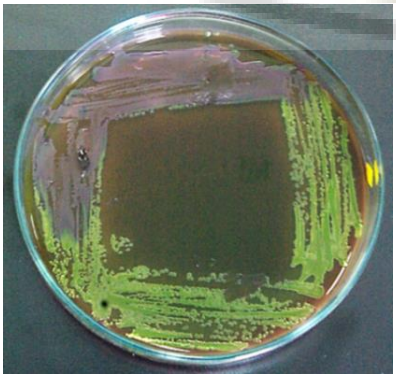


Figure 1. Single colony of *E. coli* on EMB agar with green metallic color.

III. Result

A. Bacterial Isolation

E. coli isolation was done by using EMBA selective and differential medium, the colony of *E. coli* will be shown as greenish metallic color on the agar medium (Fig. 1). From 6 places of sample, 10 *E. coli* isolate collection was isolated from 12 samples of horticulture product (Table I).

TABLE I. Isolate Collection of *E. coli*

No	Origin of Isolate	Name of Isolate
1	Traditional seller	MJ-1
2		MJ-2
3	Pagah Market	PG-1
4		PG-2
5	Patrang Market	PT-1
6	Kepatihan Market	KP-1
7	Kreyongan Market	KR-1
8		KR-2
9	Tanjung Market	PsT-1
10		PsT-2

B. Bacteriophage Isolation

The bacteriophage isolation product was found positive result on 2 samples (Fig. 2). Then both of them were named ϕPst-1 and ϕKR-1 isolate. On the medium was shown some plaque which means that the bacteria on the medium was perfectly lysed.

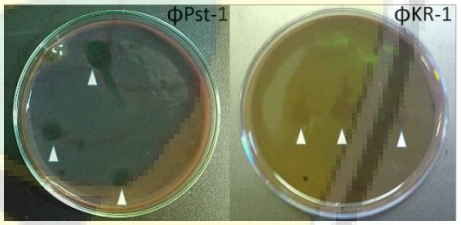


Figure 2. The Plaque were formed on EMBA medium, the differences between ϕPst-1 and ϕKR-1 plaque.

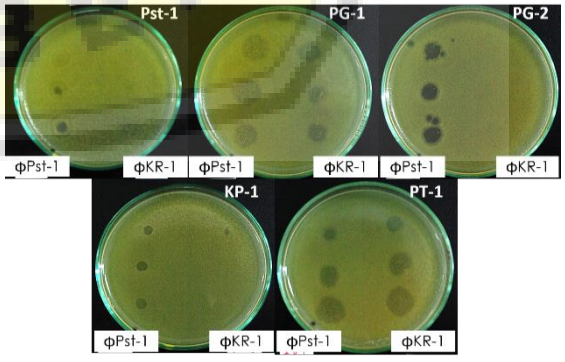


Figure 3. Host range test result by using ϕPst-1 and ϕKR-1, it prove that ϕPst-1 isolate is the most consistent isolate.

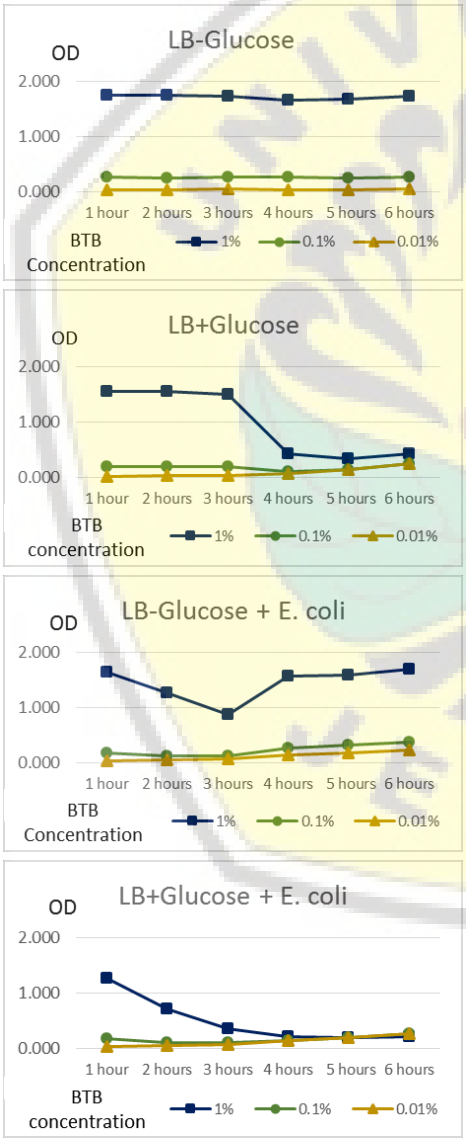
C. Host Range Test

Host range test was done on 10 isolates of *E. coli*. The host range test was done to know how will be the host

range of bacteriophage on single species of bacteria. From 10 isolates of *E. coli*, it has proven that ϕPst-1 isolate is the most consistent isolate which was able to lyse up to 5 isolates of *E. coli*, while ϕKR-1 isolate only able to lyse 2 isolates of *E. coli* (Fig. 3). Because of this result, ϕPst-1 isolate has been chosen as formulation of detection kit and will be used for the next observation.

D. Detection Kit Production

The first step to make the detection kit is concern on liquid formulation. Observation was done to see the consistency of BTB (Bromothymol blue) color by making the liquid medium mixture which was added by 0.01 %, 0.1 % and 1 % BTB also added by 1 % Glucose. The result showed that the most consistent and stable formulation color is the medium added by 0.1 % and 1 % BTB also added by 1% Glucose (Graphic 1).



Graphic 1. Detection kit formulation test result within 6 hours, medium OD consistency observation.

Solid medium screening was done by using Luria Bertani Modified Medium (LBMM). The medium which only added by bacteria without bacteriophage seen as yellow color only because the existence of *E. coli* in the medium will change the medium become a little bit acid or decrease the pH level. The pH rates changing was confirmed by BTB by color change being yellow medium. But on the other side, the green spot was appeared because it was spotted bacteriophage on the spot, so because of the existence of bacteriophage, the bacteria on the spot was lysed and failed to change the pH of the medium so the color of the medium still green. (Fig. 4)

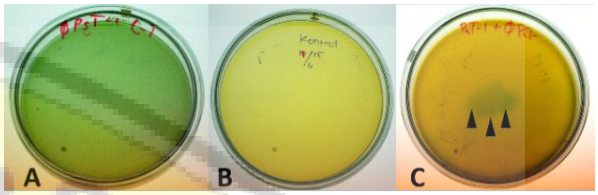


Figure 4. A). Control (LBMM), B). LBMM + *E. coli* C). LBMM+*E. coli* + ϕPst-1. Spot test result on LBMM.

The last is detection kit designing process by using the liquid medium which was added by bacteriophage. The result showed that the 1:1 comparison between the *E. coli* and bacteriophage has already shown the same result as the 1:5 comparison (Fig. 5).

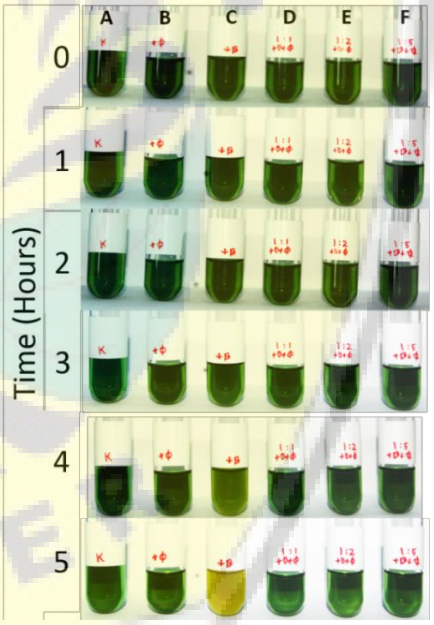


Figure 5. A) control; B) kit medium + phage(ϕ) - *E. coli*; C) kit medium - phage + *E. coli*(B); D) kit medium + *E. coli* + phage (1:1); E) kit medium + *E. coli* + phage (1:2); F) kit medium + *E. coli* + phage (1:5). Color changes of medium within 5 hours, only treatment "C" changed. "D", "E", "F" treatment still same as control (neutral pH).

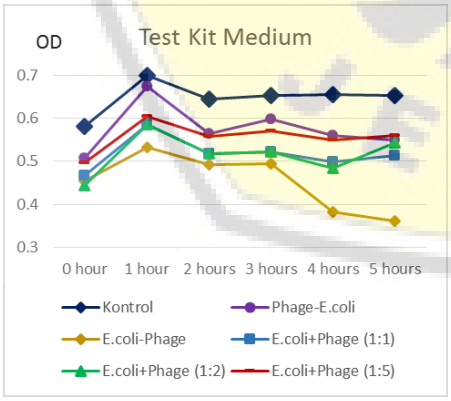
IV. Discussion

E. coli Isolates has directly isolated from some places around Jember regency. It was showed that there are similarities on colony characteristics and color on the EMBA medium. But on this research, we didn't observe the biochemical characteristic. But by the selectiveness and the differential ability provided by EMBA medium, we assume that all of the isolates are the same species which is *E. coli*.

The host range test on the *E. coli* isolates (Table I) shown that the consistency of ϕPst-1 bacteriophage isolate is better than ϕKR-1 isolate. The conclusion on this condition is although the bacteriophage have a specific host from the same species, it does not mean bacteriophage able to infect all of the strain of the host bacteria [9]. The next observation, shown that the same ϕRSS-type bacteriophage have different strain on *R Solanacearum* bacteria [10]. Ref. [11] shown that the isolated bacteriophage from *Salmonella typhi*, *P. Aeruginosa* and *E. coli* only able to infect the native host bacteria. The specificity of bacteriophage toward its host bacteria affected by protein such as lipopolysaccharide which arrange the bacterial membrane cell and it is the receptor as the adsorption of bacteriophage [9]. For example phage O4, O5, O6, and O7 have different *E. coli* host which is affected by variability of O-antigen which is the component of lipopolysaccharide [12].

The Duration of color changes of the liquid affected by the concentration of BTB. Ref. [13] addition of 0,1% BTB into the medium has been used as an indicator to differentiate the growth of bacterial colony. Ref. [14] that the bacterial growth can easily observed quickly on the medium containing BTB and carbon source including glucose. Detection kit duration is affected by the adsorption of bacteriophage. Bacteriophage able to lyse the population of *E. coli* for 15 minutes after incubation, where the most effective time to lyse the population of *E. coli* is at 120th minutes [15].

The increasing of bacteriophage multiplicity of infection (m.o.i.) toward the bacteria (1:2 and 1:5) does not differ much compared to 1:1 comparison. It indicate that bacteriophage still able to infect *E. coli* by m.o.i. 1.0 (1:1 comparison) showed by the color of the kit detection liquid which was not change at all and keep green. It proves that no change of pH rates on the liquid because the growth of bacteria can be inhibited by the bacteriophage [16].



Graphic 2. Kit medium OD changes which shown positive result on D, E, and F treatment because it is same as control.

ACKNOWLEDGEMENT

We would like to thank to the Ministry of Research and Technology Republic of Indonesia for supporting this research through the College Student Creativity Program. Besides that, we also would like to thank to Centre for Development of Advanced Sciences and Technology (CDAST) University of Jember and also Agriculture Faculty University of Jember for the supporting this paper.

REFERENCES

[1] T. F. Djaafar and S. Rahayu, "Microbial contamination on agricultural products, its pile disease and prevention," *Agriculture Research and Development*, vol. 26, no. 2, pp. 67-75, 2007.

[2] P. Yunita and U. Dwipayanti, "Microbiology quality of Nasi Jinggo based on Total Plate Count (TPC), total *Coliform* and *Escherichia coli* content," *Escherichia coli. Biology*, vol. 14, no. 1, pp. 15-19, 2010.

[3] R. Falamy, E. Warganegara, and E. Apriliana, "Detection of bacteria coliform in traditional snacks black cincau at traditional market and supermarket in Bandar Lampung City," *Majority*, vol. 1, no. 2, pp. 1-9, 2012.

[4] T. Rahayu, "The potency of isolate antibiotic of rhizosfer bacteria toward *Escherichia coli* multiresistance bacteria," *Sciences & Technology Research*, vol. 7, no. 2, pp. 81-91, 2006.

[5] Nurhayati and M. Samallo, "Rajungan (*Portunus pelagicus*) processing waste degradation analyze using commercial microbes," *Faculty of Technology Paper*, vol. 9, no. 1, pp. 1-16, 2013.

[6] M. A. Hendrayana, P. Pinatih, and A. Yelly, "Detection of bacteria *Esherichia coli* serotype o157 on pork from pork trader in denpasar city," *Medicina*, vol. 43, no. 1, pp. 3-8, 2012.

[7] A. Sulakvelidze, Z. Alavidze, and J. G. Morris, "Bacteriophage therapy antimicrob," *Agents Chemother*, vol. 45, pp. 649-659, 2001.

[8] S. Guenther, O. Herzig, L. Fieseler, J. Klumpp, and M. J. Loessner, "Biocontrol of *Salmonella typhimurium* in RTE foods with the virulent bacteriophage Fo1-E2," *Int. J. Food Microbiol*., vol. 154, pp. 66-72, 2012.

[9] A. Askora, T. Kawasaki, S. Usami, M. Fujie, and T. Yamada, "Host recognition and integration of filamentous phage φRSM in the phytopathogen, *Ralstonia solanacearum*," *Virology*, vol. 384, pp. 69-76, 2009.

[10] T. Yamada, T. Kawasaki, S. Nagata, A. Fujiwara, S. Usami, and F. Makoto, "Isolation and characterization of bacteriophages that infect the phytopathogen Ralstonia solanacearum," *Microbiology*, vol. 153, pp. 2630-2639, 2007.

[11] M. M. Sundar, G. S. Nagananda, A. Das, S. Bhattacharya, and S. Suryan, "Isolation of host-specific bacteriophages from sewage againts human pathogens," *Asian Journal of Biotechnology*, vol. 1, no. 4, pp. 163-170, 2009.

[12] W. Nimmich, U. Krallmann-Wenze, and G. Schmidt, "Bacteriophages specifically recognizing the lipopolysaccharide antigens O4, O5, O6, O7 of *Escherichia coli*," *Zentralblatt für Bakteriologie*, vol. 281, no. 4, pp. 406-414, 1994.

[13] A. A. Muhaimin, H. P. Siswanto, and W. T. D. Suryanie, "The colour differences between Escherichia coli and Pseudomonas aeruginosa colonies in artificial medium that was made from beef extract, mung beans juice, citrate and bromthymol blue," *Veterinaria Medika*, vol. 1, pp. 9-14, 2013.

[14] N. W. Schaad, J. B. Jones, and W. Chun, *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, 3rd ed., USA: APS Press, 2001, ch. 3.

[15] Carter, *et al*., "Bacteriophage cocktail significantly reduces Escherichia coli O157:H7 contamination of lettuce and beef, but does not protect against recontamination," *Bacteriophage*, vol. 2, no. 3, pp. 178-185, August 2015.

[16] R. Dhani, "The use of a bacteriophage for detector kit of bacterial blight pathogen on soybean," S.P. Script, Agriculture Department, University of Jember, Jember, Indonesia, 2014.

Febrian E. S. Iriyanto was born in Jember on February 15th, 1994. He studied in SDN Jember Lor 6 (2000-2006); SMPN 3 Jember (2006-2009); and SMAN Arjasa (2009-2012). He was studying in Agriculture Faculty University of Jember on Agrotechnology study program in Indonesia.
He is a student in University of Jember. He won National Paper Competition at Bogor Agriculture Institute on 2014 and got twice

runner-up at National Scientific Paper Competition in UNP Kediri and IKIP PGRI Madiun on 2015. He has served his paper titled "Pengendalian Penyakit Hawar Bakteri pada Tanaman Kedelai dengan Menggunakan Bakteriofag" in Trunojoyo University in 2014.

Wahyu C. Yuliasari was born in Jember on July 6th, 1993. She studied in SDN Dukuh Dempok 1 (2000-2006); SMP "Plus" Darus (2006-2009); and SMAN 3 Jember (2009-2012). She was studying in Agriculture Faculty University of Jember on Agrotechnology study program in Indonesia.
She is a student in University of Jember. She has served his paper titled "Pengendalian Penyakit Hawar Bakteri pada Tanaman Kedelai dengan Menggunakan Bakteriofag" in Trunojoyo University in 2014.

Arie Rahmawati was born in Madiun on February 17th, 1994. She studied in SDN Kebonagung 2 Mejayan (2000-2006); SMPN 2 Mejayan (2006-2009); and SMAN 1 Mejayan (2009-2012). She was studying in Agriculture Faculty University of Jember on Agrotechnology study program in Indonesia.

Muhammad Sholehuddin was born in Bondowoso on September 22nd, 1994. He studied in SDN Taman 2 (2001-2007); SMPN 1 Bondowoso (2007-2010); and SMAN 2 Bondowoso (2010-2013). He was studying in Agriculture Faculty University of Jember on Agrotechnology study program in Indonesia. He is a campus ambassador of University of Jember. He got an award as the best oral presentation on the 3rd International Conference on Agriculture and Biotechnology (ICABT 2015), Jinju, South Korea.

Angga A. Ramadhani was born in Jember on February 15th, 1995. He studied in SDN 5 Rambipuji (2001-2007); SMPN 1 Rambipuji (2007-2010); and SMKN 5 Jember (2010-2013). He was studying in Agriculture Faculty University of Jember on Agrotechnology study program in Indonesia.

Hardian S. Addy was born in Manokwari on September 11th, 1980. He studied in University of Jember, Indonesia (1999-2003) in plant protection majority (S.P); Gajah Mada University, Indonesia (2003-2005) in fitopatology majority (M.P); and Hiroshima University, Japan (2009-2012) in molecular biotechnology majority (PhD).
He is a researcher in Centre for Development of Advanced Science and Technology (CDAST) on University of Jember. He is a lecturer also in Agriculture Faculty University of Jember. He has published 12 journals and one book which are: Association of filamentous phage with *Ralstonia solanacearum*: Molecular study on ϕRSM3 and ϕRSS1 (LAP LAMBERT Academic Publishing: Hardian S. Addy, 2012. Currently he is concern on bacteriophage research.
Hardian Susilo Addy, S.P., M.P., Ph.D. achieved an award given by ministry of research and technology of Indonesia as the best national researcher and presenter in 2005, Travel Grant award to Foreign Student for International Meeting and Conference Abroad at Hiroshima University in 2010, Scheme for Academic Mobility and Exchange 2014 at Hiroshima University, Runner-up of the outstanding luecturer of University of Jember 2015, and Short-course Grant awardee for Biotechnology Regulation Immerssion Course, Unviversity of Missouri, Columbia in 2015.