AJM BES Vol. 20, No. 2, 2018 (ISSN 0972-3005)

Asian Journal of Microbiology, Biotechnology & Environmental Sciences

MICROBIOLOGY

BIOTECHNOLOGY

ENVIRONMENTAL SCIENCES

Editors
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Global Science Publications, India

Asian Journal of Microbiology, Biotechnology & Environmental Sciences (AJMBES)

Quarterly International Journal (ISSN 0972-3005)

ABSTRACTED IN : Chemical Abstracts, USA, Ecological Abstracts, Cambridge Science Abstracts, Ecodisc CD-ROM, Geological Abstracts, Pollution Abstract, International Development Abstracts, (Elsevier), Paryavaran Abstracts India, Indian Science Abstracts, Current Awareness in Biological Sciences, Word Textile Abstracts Fluid Abstracts, Oceanography Literature Review etc., Covered in SCOPUS (Elesevier's).

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ASIAN JOURNAL OF MICROBIOLOGY, BIOTECHNOLOGY AND ENVIRONMENTAL SCIENCES
(VOL. 20 NO. 2, 2018)

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Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 20 (2) : 2018 : 439-444

ISSN-0972-3005

ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGE IN CONTROLLING *Escherichia coli* IN JEMBER AREA, INDONESIA

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(Received 16 October, 2017; accepted 1 December, 2017)

Key words: *Bacteriophage, Escherichia coli, Host range, Protein profile*

Abstract – *Escherichia coli* contributes to the contamination of horticultural products,the prevention of that bacterial contamination can be carried out using bacteriophage. This research is aimed to determine the characteristics of bacteriophages and their interactions with host bacteria. Characteristics of isolated *Escherichia coli*, morphology of bacteriophages capable of *Escherichia coli* infection and host range of that particular bacteriophage were observed. The result revealed that all the bacteriophages obtained namely ϕIGKR1a, ϕIGKR1b, ϕIGKR2, ϕIGPT1a, ϕIGPT1b and ϕIGMJ1b belong to the group of *Myoviridae*. Isolated *Escherichia coli* namely KR, KP, MJ, PR and PT were used as host bacteria. The host range test showed that all bacteriophages could infect KR, KP and MJ bacteria. Bacteriophage characteristics can be seen from the protein profile. The six bacteriophages had similar protein profile and bands size. The thick bands formed on SDS gel showed the bands of the major proteins and the thin ones were minor proteins.

INTRODUCTION

Food apart of its main function as source of nutrients can be the cause of certain diseases. Foodborne disease caused by consuming contaminated food and beverages. Vegetable and fruits are the most vulnerable food ingredients prone to contamination (Yang *et al.*, 2010). Vegetables can carry microorganisms such as bacteria, both pathogenic and non-pathogenic bacteria. The main bacteria responsible for food contamination is *Escherichia coli*. *Escherichia coli* is a gram-negative rod-shaped bacteria belonging to the Enterobacteriaceae family, which means this bacteria can live in the gastrointestinal tract. *Escherichia coli* can be pathogen due to the ability to produce diarrhea-caused enterotoxin compound (Brooks *et al.*, 2004).

The treatment to cure food borne disease caused by *Escherichia coli* was the administration of antibiotics, but several studies found that *Escherichia coli* has been resistant to some antibiotics (penicillin, tetracycline and erythromycin) (Pourtaghi *et al.*, 2016; Rasmussen *et al.*, 2015; Samms *et al.*, 2015). Based on this finding, the need for another treatment alternative is required. One of the proposed solution is by using natural enemies such as bacteriophage. Bacteriophages are viruses that attack bacteria. Bacteriophages work specifically to recognize their host (Sulakvelidze, 2013). The specific characteristics of these bacteriophages can be utilized in characterizing bacteriophages that infect *Escherichia coli*. Isolation and bacteriophage characterization had been performed on the isolation and characterization of Campylobacter bacteriophages from retail poultry (Atterbury *et al.*, 2003). Research on bacteriophages in Indonesia is still rare, especially research on the isolation and characterization of bacteriophage for *Escherichia coli* causing foodborne disease. Hence, study on isolation, characterization and exploration efforts of *Escherichia coli* bacteriophage is essential.

In this study, we reported the characteristics of six bacteriophages from Jember-Indonesia infecting *Escherichia coli.*

MATERIALS AND METHODS

Isolation of *Escherichia coli*

Escherichia coli was isolated from various vegetables from five traditional markets in Jember (Table S1).

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The bacteria were isolated from 10^{-3} diluted dishwater of vegetable and then grew on Eosin Methylene Blue Agar (EMBA) using spread plate method. The single colony of bacteria then transfered to agar slant containing EMBA medium using streakmethod and incubated for 12 up to 24 hours at 37°C.

Isolation and purification of bacteriophage

Table 1. Identity of *Escherichia coli* bacterial isolates from four markets and traditional seller in Jember-Indonesia

No	Isolate of *Escherichia coli*	Source of vegetable
1.	KR	Kreongan Market
2.	MJ	Traditional Seller
3.	KP	Kepatihan Market
4.	PT	Tanjung Market
5.	PR	Patrang Market

The bacteriophages were isolated from dishwater of vegetables from five traditional markets in Jember. Then, 30 mL dishwater and 100µL of bacterial culture mixed on LBM. The incubationprocess took for 24 hours. After incubation, those bacteriophage were filtered by 0,2 µm membrane filter. The filtrate was the bacteriophage isolate and will be used to spot test.

Host range of bacteriophage

The host range test was held by spot test method (Armon and Kott, 1993). First, the bacteria were culturedin Luria Bertani Medium (LBM) and incubated at 37°C. Incubated bacterial cultures were mixed in the Top Agar (0.6% agar) of 200 µL and poured in Luria Bertani Agar medium. The double layer was left for about 30 minutes and then did spot test with several bacteriophages to measure the host range. Each bacteriophage was dispersed as much as 2 µL.

Protein profile of bacteriophage infecting *Escherichia coli*

The protein profile of bacteriophage usingmethod by Addy and Wiwiek (2016). Purification of bacteriophage protein conducted using micro ultracentrifuge 40.000 rpm for 2 hours and temperature 4°C. The pellet is mixed with saline phosphate buffer and the bacteriophage protein that has been subsequently obtained in SDS-PAGE and stained with Coomasie Briliant Blue.

RESULTS

Isolation of *Escherichia coli*

Isolation of *Escherichia coli* using spread plate method produced many colonies in different shapes. The colony of *E. coli* has a blackish colored which are then transfered for purification. The purification continue until a uniform colonies were obtained. *Escherichia coli* appeared blackish or green metalic on EMBA medium (Hendrayana, *et al.*, 2012). The specific color is formed due to precipitation of methylen blue. Lactose fermentation in the medium resulted in decreasing pH, this acidic condition will induce methylen blue to precipitate (Cheeptham, 2012). The isolation obtained 5 types of *Escherichia coli* namely KR, MJ, PT, KP, and PR (Table S1).

Isolation and purification of bacteriophages

Bacteriophage isolated from dishwater of vegetable and the presence of bacteriophages will be demonstrated by plaque (Irianto *et al.*, 2016). The plaque formed then purified by using plaque assay to obtain a single plaque. The result of bacteriophage isolation was 6 strains namely ϕIGKR1a, ϕIGKR1b, ϕIGKR2, ϕIGPT1a, ϕIGPT1b and ϕIGMJ1b (Fig. 1) and the plaque showed cloudily. Bacteriophages can also appear clear plaque as in ϕRSB2a, ϕRSB2, ϕRSB3, ϕRSJ2 and ϕRSJ5 which infect *Ralstonia solanacearum* (Kawasaki, *et al.*, 2016). Bacteriophages of ϕIGPT1a, ϕIGPT1b, ϕIGMJ1b observed their morphology by using transmission electron micrographs. Bacteriophage of φIGPT-1a has icosahedral head with ± 70 nm in diameter; tail length ± 50 nm and tail width ± 10 nm. While, bacteriophage of φIGPT-1b has icosahedral head with diameter ± 120 nm; tail length ± 200 nm and tail width ± 9 nm. Bacteriophage φIJ1b has icosahedral head with diameter ± 90 nm; tail lenght ± 100 nm and tail width ± 20 nm (Fig.2).

Host range and protein profile of bacteriophages that infected *Escherichia coli*

The host range was performed on five isolates of *Escherichia coli* to determine the host range of each bacteriophage in each isolated bacterial isolate. The result of the host range showed that the six bacteriophages are able to infect three bacterial isolates, namely KR, KP and MJ isolates which are shown by the formation of plaques. The plaques

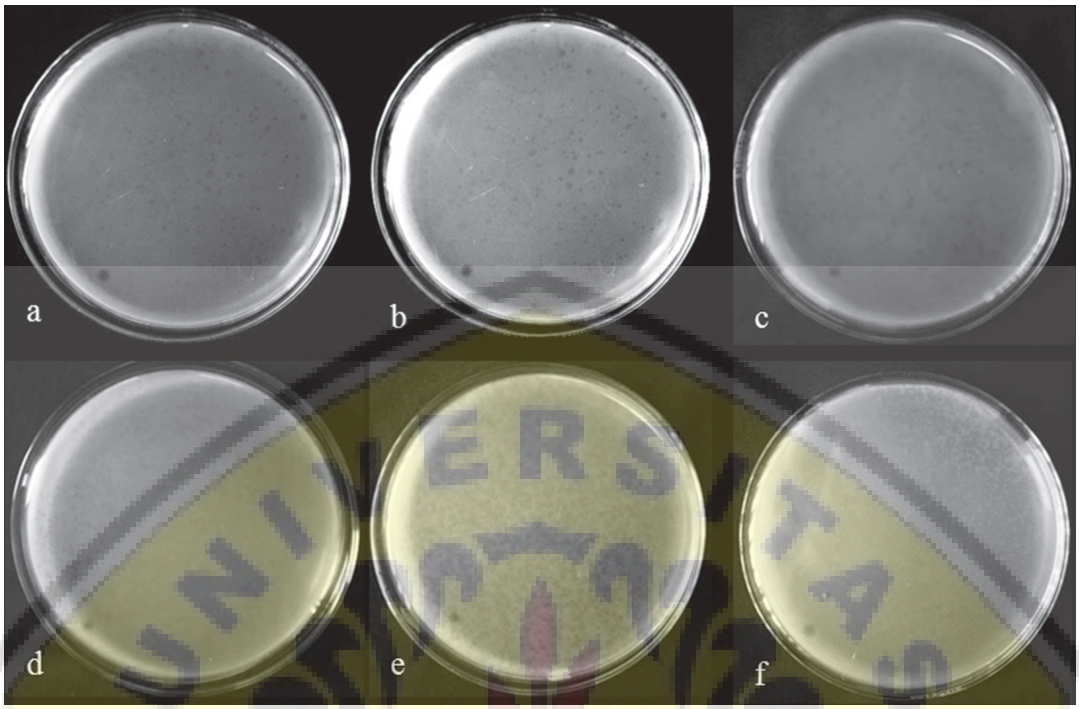

Fig. 1. Different plaque morphologies indicate the presence of bacteriophage (a) ϕIGKR1a, (b) ϕIGKR1b, (c) ϕIGKR2, (d) ϕIGPT1a, (e) ϕIGPT1b, (f) ϕIGMJ1b

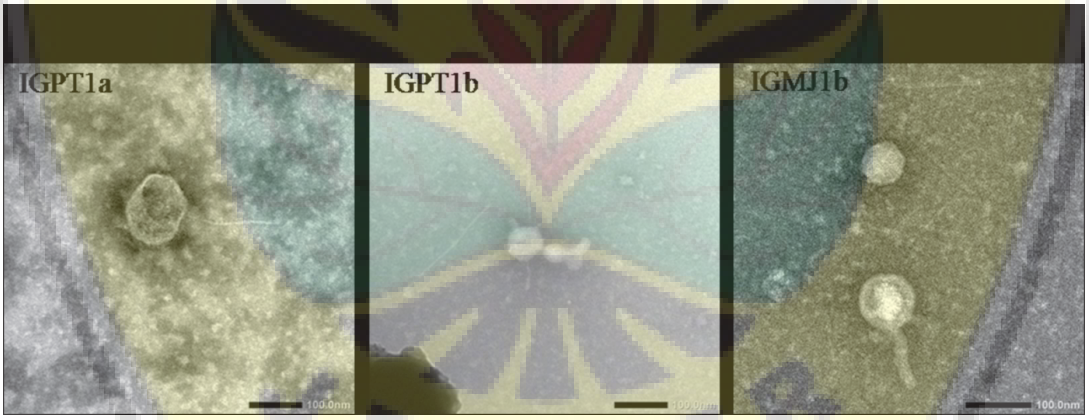

Fig. 2. Electron micrographs of bacteriophages isolated from dishwater of vegetables.

that form on each bacterial isolate have different turbidity levels. In MJ, bacteriophage isolates have strong infection towards ϕIGKR1a, ϕIGKR1b, ϕIGPT1b and ϕIGMJ1b, whereas ϕIGKR2 and ϕIGPT1a could infect with a thin plaque yield. Isolated bacteria PT and PR, the results indicated there were no bacteriophages that could infect both bacterial isolates (Fig. 3). Host range results showed that although bacteriophages have a certain host of the same species, it does not mean that bacteriophages are capable of infect all the strain of the host bacteria (Askora *et al.*, 2009; Narulita, *et al.*, 2016).

Bacteriophages can also be recognized by profile protein they have by using SDS-PAGE (SDS-Polycrylamide gel electrophoresis). In this study, the profile protein of the six different bacteriophages showed similar protein profile (Fig. 4). The polyacrylamide gel showed that there are several protein bands in one type of bacteriophage. According to Black and Rao (2012). and Rao that viral proteins or bacteriophages can be composed of several sub-units of proteins. The protein component in bacteriophage consists of several

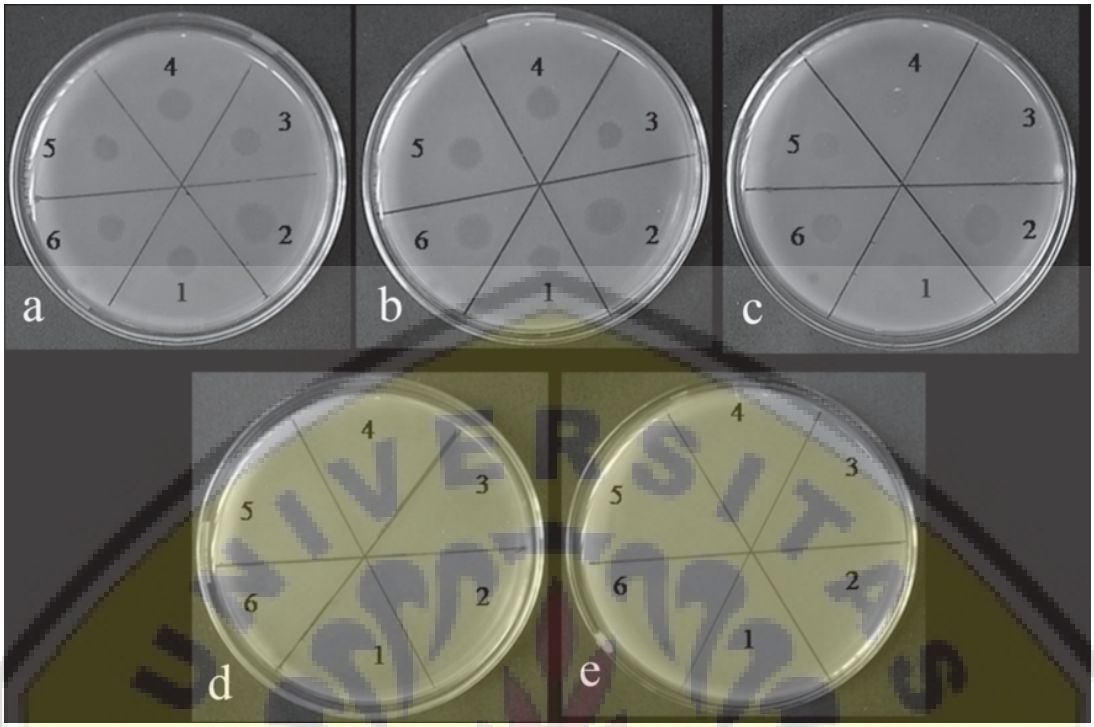

Fig. 3. Host range of six bacteriophages (1) ϕIGKR1a, (2) ϕIGKR1b, (3) ϕIGKR2, (4) ϕIGPT1a, (5) ϕIGPT1b, (6) ϕIGMJ1b against five bacterial isolates *E. coli* (a) KR, (b) KP, (c) MJ, (d) PT and (e) PR.

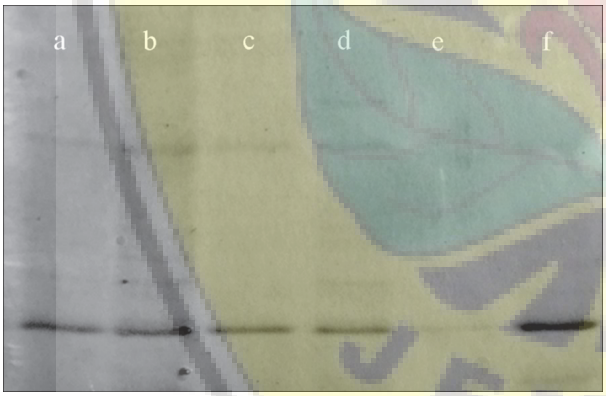

Fig. 4. Protein pattern of bacteriophage analyzed with SDS-PAGE (12.5%).

proteins including the major protein capsid, tail caudal protein, tail protein (Kwiatek *et al*., 2012).

DISCUSSION

Escherichia coli is directly isolated from four traditional markets around Jember Regency and traditional seller which have been used as vegetable samples. Vegetables that have been isolated showed positive result of *Escherichia coli*. In the EMBA media, *Escherichia coli* colonies will be shown green metallic morphology caused by *E. coli* lactose fermentation, causing an increase in acid levels in the media and caused methylen blue precipited in the medium that produces color green metallic in the colony of *Escherichia coli* (Hassan *et al.,* 2015). The colonies of *Escherichia coli*on EMBA medium were round and slimy with blackish color of the colony, this is in accordance with (Baehaqi *et al.,* 2015). Based on the identification of *E. coli* colonies on EMBA medium showed that bacterial colonies that have been isolated showing the characteristic of *E. coli* colonies.

The six types of isolated bacteriophages have similar size and shape, but different levels of turbidity in each type of bacteriophage. The compatibility of bacteriophage when infecting receptor molecules on the surface of the host cell will affect the formation of the plaque. Bacteriophages will not show interactions with receptors that have different structures [12]. Different sizes on each plaque produced by bacteriophages are caused by different types of bacteriophages. Bacteriophages entering the *Myoviridae* family will produce a small plaque while the *Siphoviridae* and *Podoviridae* families will produce a larger plaque (Haq *et al.,* 2012).

The isolate of bacteriophages have a small plaque size, so it was possible that these bacteriophages were belong to *Myoviridae* family. Different forms of plaque can occur due to several reasons such as decreasing lytic level of bacteriophage in infecting host cells (lysation inhibition phenomenon), bacteriophage that produce certain enzymes to destroy bacterial envelope cells to form a plaque that has a different appearance (Jurczak-Kurek *et al*., 2016). The levels of opacities different plaque may indicate the cycle type of bacteriophage itself. Bacteriophages that have clear plaque may be indicated that the bacteriophage is lytic bacteriophage (virulent). When it was murky plaque, it may indicate the type of lysogenic bacteriophage (temperate) present in the plaque. The six isolated bacteriophages have a murky plaque and the plaque indicates a temperate bacteriophage.

The host range tests of ϕIGKR1a, ϕIGKR1b, ϕIGKR2, ϕIGPT1a, ϕIGPT1b and ÕIGMJ1b, indicated that from all 6 bacterial isolates only three isolates capable of being infected by bacteriophage. Such circumstances can occur because of several possibilities, such as the incompatibility of binding proteins in bacterial receptors and bacteriophages so as to avoid infection because bacteriophages work specifically against their host. Bacteriophages are host specific, they unable infect other human and eukaryotic cells (Grygorcewicz *et al*., 2015). Another possibility that may occur was the presence of bacterial isolates that were resistant to phage (Golkar *et al*., 2014). Bacteria isolate of PT and PR that resistant to phages infection might cause of its lack of a suitable receptor.

Bacteriophage characteristics can be seen from the pattern of protein possessed. The protein pattern formed on the 12.5% SDS gel that has been performed shows that the six bacteriophage bands have almost the same size and pattern, which are at the bottom of the gel. The bands that form thickly on the SDS gel shows the bands of the major proteins and the thin-looking ones are minor protein bands. Total amount of protein bands in gel were four major protein bands and six minor protein bands. In this study, the SDS gel showed the presence of one major protein bands and about one to two minor protein bands seen in each sample. Such a situation occurs because the amount of protein extracted in each sample of bacteriophage is too small so that the results obtained less than the maximum (Grygorcewicz *et al*., 2015).

Conflict of interest

The authors declare no conflict of interests.

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