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

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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 570 nm. Detection assay was also done using mixture of detection solution, sample, and bacteriophage ϕPsT-1 which is 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 10⁻³ 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.
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).

<p>| TABLE I. ISOLATE COLLECTION OF E. COLI |
|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>No.</th>
<th>Origin of Isolate</th>
<th>Name of Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Traditional seller</td>
<td>MJ-1</td>
</tr>
<tr>
<td>2</td>
<td>Pagah Market</td>
<td>PG-1</td>
</tr>
<tr>
<td>3</td>
<td>Patrang Market</td>
<td>PT-1</td>
</tr>
<tr>
<td>4</td>
<td>Kepatihan Market</td>
<td>KP-1</td>
</tr>
<tr>
<td>5</td>
<td>Kreyongan Market</td>
<td>KR-1</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>KR-2</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>PsT-1</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>PsT-2</td>
</tr>
</tbody>
</table>

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.

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).

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)

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).

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 \textit{E. coli} isolates (Table I) shown that the consistency of $\phi$Pst-1 bacteriophage isolate is better than $\phi$KR-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 \cite{9}. The next observation, shown that the same $\phi$RSS-type bacteriophage have different strain on \textit{R. Solanacearum} bacteria \cite{10}. Ref. \cite{11} shown that the isolated bacteriophage from \textit{Salmonella typhi}, \textit{P. Aeruginosa} and \textit{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 \cite{9}. For example phase O4, O5, O6, and O7 have different \textit{E. coli} host which is affected by variability of O-antigen which is the component of lipopolysaccharide \cite{12}.

The Duration of color changes of the liquid affected by the concentration of BTB. Ref. \cite{13} addition of 0.1\% BTB into the medium has been used as an indicator to differentiate the growth of bacterial colony. Ref. \cite{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 \textit{E. coli} for 15 minutes after incubation, where the most effective time to lyse the population of \textit{E. coli} is at 120\% minutes \cite{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 \textit{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 \cite{16}.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2}
\caption{Kit medium OD changes which shown positive result on D, E, and F treatment because it is same as control.}
\end{figure}

\section*{ACKNOWLEDGEMENT}

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