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Comparation Between Mac conkey and Coconut Water Medium as a Growth Medium for *Escherichia coli*

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Abstract

Escherichia coli is the bacteria that can cause diarrhea in humans and often used as a parameter of stool environmental pollution. Culture of *E. coli* from the sample often requires Mac Conkey as commercial media which is able to distinguish it from other bacteria in the Enterobacteriaceae group. Commercial media such as Mac Conkey certainly has a price that is quite expensive because of its ability as a growth medium for Enterobacteriaceae. Therefore, in the study tested natural ingredients that can be used for growth media, such as coconut water. The purpose of this study was to compare the ability of Mac Conkey media and coconut water to support the growth of *E. coli*. This research is an experimental study with a completely randomized design. The concentration of coconut water tested was 0%, 20%, 40%, 60%, 80%, and 100%. The results showed that at the concentration of coconut water 20% to 60% the number of *E. coli* colonies on coconut water media was slightly below the Mac Conkey Agar media, while in coconut water a concentration of 80% showed a greater number of colonies than Mac Conkey. The Mann Whitney test showed a significant difference between the number of colonies on 80% coconut water media and Mac Conkey Agar, which was equal to 0.004 ($p < 0.05$). Based on these results, coconut water has the potential to be used as a growth medium for *E. coli*.

Keywords

Coconut Water, *Escherichia coli*, Mac Conkey.

INTRODUCTION

Indonesia is an archipelago which produces coconut fruit in most of provinces (1). Indonesia is one of country in the world which produces a high number of coconut fruit per year. Coconut were being consumed for its water and flesh, and or its product as kopra and coconut oil (1). In coconut water contain carbohydrate, protein, fat, vitamin, and minerals (2,3). Coconut water can be used for a body electrolytes subtitution, antidotes, antioxidants, to antibacterial beside for being consumed (4,5,6). Coconut water can also be used as a medium for making food products such as nata de coco (7, 8). Some traditional markets in Indonesia sell coconut meat and often not use the water. Abundance and ease of the access to obtain coconuts can be used as opportunities for wider use of coconut water. Utilization of coconut water for bacterial growth media in the laboratory is one of the ways to access it. The examination parameter which often carried out in the laboratory is the examination of *Escherichia coli*.

E. coli is the bacteria that can cause diarrhea in humans (9, 10, 11) and often used as a parameter of stool environmental pollution (12, 13, 14). Culture of *E. coli* from the sample often requires Mac Conkey as commercial media which is able to distinguish it from other bacteria in the Enterobacteriaceae group. Commercial media such as Mac Conkey certainly has a quite expensive price because of its ability as a growth medium for Enterobacteriaceae. Therefore, in this study were tried to compare viable count of *E. coli* recovered from Coconut water medium and Mac Conkey as gold standard for growing *E. coli*.

MATERIALS AND METHODS

This study was an experimental study using complete randomized design. Variable of this study were coconut water concentration (0%, 20%, 40%, 60%, 80%, 100%) and numbers of *E. coli* colony. This study conducted in Microbiology Laboratorium at Universitas Nahdlatul Ulama Surabaya, Indonesia.

This study used *E. coli* ATCC 25922 that were obtained from BBLK (*Balai Laboratorium Kesehatan*) Surabaya. *E. coli* need to subculture in nutrient agar slant before used. Twenty-four bacterial culture in nutrient agar slant then suspend in sterilize 0.85% natrium chloride until it reaches Mac Farland 0.5 turbidity.

Coconut water between 6 and 8 months of age were obtained from traditional market. Coconut water was extracted by breaking coconut endocarp.

Coconut water obtained then analyse for chemical characters, namely carbohydrate, protein and fat component in *Balai Riset dan Standarisasi Industri Surabaya* (Baristand Industri Surabaya) Indonesia. Composition of the coconut water media per 100mL were

lactose 10 g; agar 1.5 g; neutral red 2% 2 mL; aquadest (varying concentration of the coconut water); Coconut Water (0%, 20%, 40%, 60%, 80%, 100%). Each of media component were mixed and boiled to allow completely dissolve. Media was sterilized using autoclave for 15 minutes at 121^{o}C, 1 atm.

For pure *E. coli*, a uniform cell suspension was pepared by dispersing the overnight colony growth from nutrient agar media. The turbidity of the bacterial suspension used was according to the Mc Farland 0.5 standard (0.05 mL of 1.175% barium chloride dihydrate ($BaCl_2.2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4)). Bacterial suspension was inoculated using pour plate method with a proper dilution. Bacterial suspension diluted in series of 0.85% Natrium Chloride. Each of serial dilution bacterial suspension were taken as much as 1 mL and placed it in sterile petridish. Media were poured to sterile petri dish and left to solid. Media were in icubated at 37^{o}C for 24 hours in incubators. The replication used in the study was 5 times for each treatment.

The result of this work was observation of the the number of colonies-forming units and its macroscopic colonies of *E. coli*. Numbers of *E. coli* (CFU) obtained from medium after incubation then analyze statisticly using mann whitney. Colony morphology was illustrated in diagram.

RESULTS

This study used young coconut water for growth medium of *E. coli* and comparing it with Mac Conkey as commercial medium. Base component of coconut water used were illustrated in Table 1.

The bacterial colony recovered in each group has vary in its colony size. *E. coli* growth in Mac Conkey show medium category in colony size while in coconut water medium show vary colony size from small pin point category until small colony category (Figure 1). Bacterial colony number which recovered in each group of coconut water medium treatment shows slightly increasing in colony number while number of *E. coli* colony at 80% group were exceed Mac Conkey group (Figure 2). Colony colour in coconut water group were white while colony in Mac Conkey were red (data not shown).

Table 1. Composition per 600 mL of young coconut water

Componet	Percentage
Protein	0.453
Carbohydrate	4.488
Fat	0.257

DISCUSSION

Coconut water contain numerous nutrition components for bacterial growth, including macro and micro component (15, 16). There are various types of coconuts, with different mineral content in coconut water. Chuku & Calagbour (16) compared various coconut species for its coconut water content

which are mineral and protein content. In general, the largest to lowest mineral content in a row were Cl - K - Na - Ca - S - Fe - Cu. The mineral components in coconut water are needed for bacterial growth. In addition to minerals, coconut water also contains sucrose, glucose, and fructose (15). The presence of these sugar in coconut water createthe colony of *E. coli* bacteria can not express pink color at 24-hour incubation, which indicates the absence of lactose fermentation in coconut water media. *E. coli* tends to use the simplest sugar available in the media first before complex ones (17). *E. coli* colony shows discoloration change to pink after one week of incubation (data not shown). In this study, the 80% *E. coli* treatment group had a large count of colony growth but has smaller colony size compared to growth using Mac Conkey media. This condition is possible because in medium coconut water has a low source of nitrogen and phosphorus. Limitation of phosphorus in the media will slowing the translation process because of the low certain ribosomes and the RNA is very much so that it requires a lot of phosphorus. Meanwhile, nitrogen limitation cause a slow elongation process of the translation process (18).

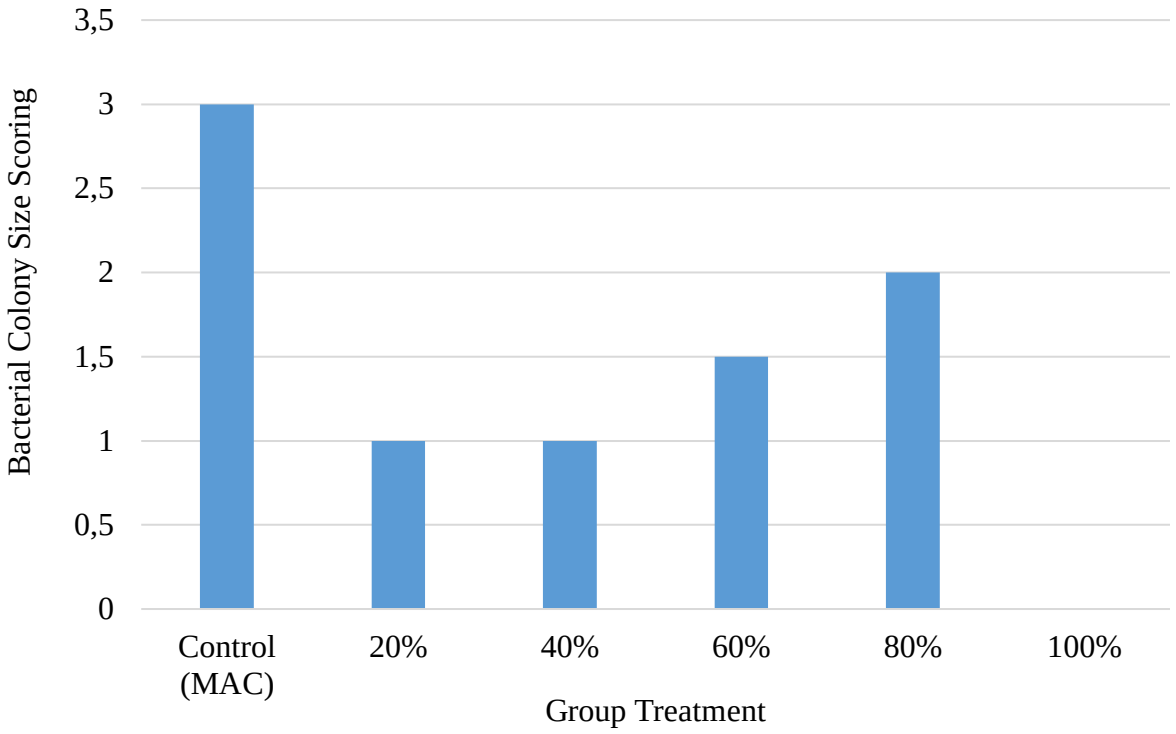

Figure 1. *Escherichia coli* colony size scoring in each of group treatment. Treatment group: control using Mac Conkey Agar and coconut water media agar by adding coconut water in media approximately 20%, 40%, 60%, 80% and 100%. Growth of *E. coli* in each treatment then scored based on size, score 3 for medium size; 2 for small size; 1.5 for pin point size; 1 for small pin point size; 0 for no growth.

The differences between both testing media were absence of nitrogen source, bile salt, and crystal violet in the coconut water medium. One of Mac Conkey media component are peptone, in which can promote enrichment for bacterial colony. Peptone is nitrogen source for bacteria, and oftenly used as enrichment purpose (19).

The Phytochemical testing of several types of coconut water qualitatively shows the presence of tannin content in coconut water. Tanin is one of phytochemical which can inhibit bacterial growth (20). It explains no *E. coli* growth in 100% group.

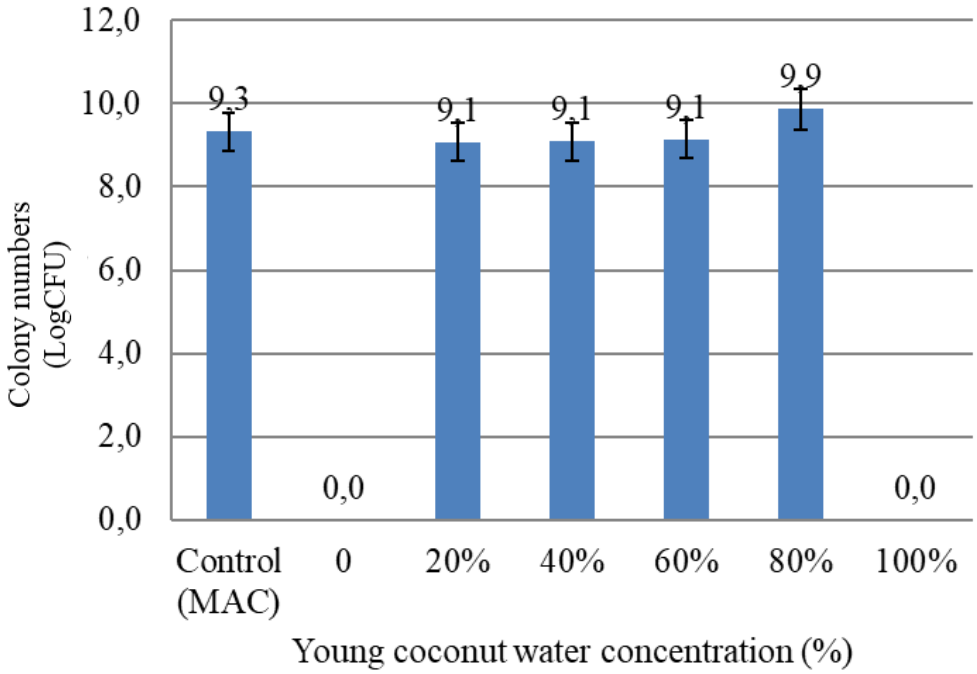

Figure 2. Numbers of *Escherichia coli* Colony Recovered in Mac Conkey and coconut water Media. Treatment group: control using Mac Conkey Agar and coconut water media agar by adding coconut water in media approximately 20%, 40%, 60%, 80% and 100%.

CONCLUSIONS

Coconut Water ia able to use as component medium for the *E. coli* cultivation. The coconut water has a good source for carbohydrate. Further evaluation needs to be carried out to explore the coconut water physiological influenced bacterial growth.

AUTHOR CONTRIBUTIONS

Endah Prayekti: conceptualization, methodology, writing-original draft, visualization, supervision, funding

acquisition. Suliati: methodology, supervison. Dwi Agustin Wulandari: formal analysis, investigation, resources.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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