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Date Palm (*Phoenix dactylifera L.)* Flour as an Alternative Culture Media for the Growth of *Escherichia coli* and *Bacillus cereus*

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Abstract

The high cost of bacterial growth culture media can result in obstacles the process of practicum or research in the field of microbiology. Date palm (*Phoenix dactylifera* L.) is the oldest fruit plant that grows in the Arabian Peninsula, North Africa and the Middle East. Dates are a source of high energy food with sugar content of 72% - 88%. This research was conducted to test the ability of date palm flour to grow *Escherichia coli* and *Bacillus cereus* bacteria as an alternative medium to replace NA media. In this study using the method experimental laboratories with concentration 1 g, 2 g, 4 g, 6 g, 8 g with three repetitions. The results of this study the number of colonies of *Escherichia coli* bacteria was more than the number of colonies of *Bacillus cereus*, that is 54 x 10^5CFU/g on concentration media 8 gr. When compared with NA media, date palm flour media can be used as an inexpensive alternative culture medium for bacteria.

Keywords: Bacillus cereus, Escherichia coli, alternative media, date palm flour

Original Research Article

Tepung Kurma (Phoenix dactylifera L.) sebagai Media Kultur Alternatif Pertumbuhan Bakteri Escherichia coli dan Bacillus cereus

Abstrak

Mahalnya biaya media kultur pertumbuhan bakteri berakibat dapat menghambat dalam proses praktikum atau penelitian bidang mikrobiologi. Kurma (Phoenix dactylifera L.) merupakan tanaman buah tertua yang tumbuh disemananjung Arab, Afrika utara dan Timur tengah. Buah kurma merupakan sumber makanan yang berenergi tinggi dengan kadar gula 72% - 88%. Penelitian ini dilakukan untuk menguji tepung kurma mampu menumbuhkan bakteri Escherichia coli dan Bacillus cereuss sebagai media alternatif pengganti media NA. Dalam penelitian ini menggunakan metode eksperimental laboratorium menggunakan konsentrasi 1, 2, 4, 6, dan 8 gram dengan tiga kali pengulangan. Hasil dari penelitian ini jumlah koloni bakteri Escherichia coli lebih banyak daripada jumlah koloni Bacillus cereus yaitu 54 x 10^5 CFU/g pada konsentrasi media 8 gram. Bila dibandingkan dengan media NA, media tepung kurma dapat dijadikan media kultur alternatif bakteri yang murah.

Kata Kunci: *Bacillus cereus, Escherichia coli, media alternatif, tepung kurma*

INTRODUCTION

Microorganisms need nutrients as a source of energy and certain environmental conditions to grow and reproduce. Culture media is a substrate that bacteria can use to grow and reproduce. Bacteria can grow and reproduce if the culture media contains nutrients such as carbon, nitrogen, inorganic salts such as sulfate, folate, potassium,

sodium magnesium, calcium, iron, manganese. Bakteria *Escherichia coli* and *Bacillus cereus* are pathogenic bacteria that are often the causative agents foodborne disease (Wang et al., 2015). Clinical symptoms of contamination by *Escherichia coli* and *Bacillus cereus* such as poisoning, vomiting, diarrhea (Sumampouw, 2018). These bacteria can be grown on universal media that are often used such as media NA (*Nutrient Agar*) because contain simple molecules such as ammonia and carbon dioxide needed by bacteria (Olivia, 2017). In developing countries, the high price of culture media often inhibits microbiology practicum or scientific research in institutions. Commercial media that is often used in the laboratory is *Nutrient agar* media (NA) in the market the price of Na media is around Rp. 1 million-3 million per 500 grams. NA media contains beef extract, peptone which is a source of protein, nitrogen, vitamins, carbohydrates which are needed by bacteria to grow and develop. The high price of bacterial culture media encourages research on alternative culture media that are cheap at affordable prices by utilizing local raw materials derived from plants, vegetables, fruits such as potatoes, cereals, cassava, soybeans, carrots, cabbage) (Deivanayaki and Antony, 2012), media alternative from rice, corn, soybean flour for the growth of *Klebsiella*, *Pseudomonas* sp, *Bacillus* sp (Uthayasooriyan et al., 2016), vegetable waste can be potential as a culture media for growth bacteria and yeast such as *Bacillus* sp, *Pseudomonas aeruginosa, Candid albicans, Saccharomyces cerevisae* (Berde and Berde, 2015).

Date palm (*Phoenix dactylifera* L.) is the oldest fruit plant that grows in the Arabian Peninsula, North Africa and the Middle East. Date palms produce many useful products for humans. Dates can be eaten fresh, dried or in processed forms such as cereals, puddings, breads, pastries, candies, ice cream and can also be added to juices, syrups, honey. Dates have high nutrition and have many benefits such as antifungal, antidiarrheal, antioxidant, anti-inflammatory (Mallhi et al., 2014). Dates are a source of high energy food with sugar content of 72% - 88% when the dates are ripe. Date fruits have a high nutritional being rich in carbohydrates, proteins, minerals, vitamin B complex such as thiamine (B1), riboflavin (B2), niacin (B3), pantothenic (B5), pyridoxine (B6), folate (B9) (Al-Harrasi et al, 2014). Phytochemically date palm contains alkaloids, flavonoids, anthraquinone, saponin, terpenoids and tannin (Oni et al, 2015). *Escherichia coli* and *Bacillus cereus* are pathogenic bacteria that cause infection foodborne with diarrhea symptoms. Microorganisms cultured on date flour media are *Escherichia coli* which is a gram-negative bacteria and *Bacillus cereus* is a gram positive bacteria. So far, studies on the use of date palm flour as an alternative medium have not been studied. This study aims to test whether date flour is able to grow *Escherichia coli* and *Bacillus cereuss* bacteria as an alternative medium for replace NA media (Gharib et al., 2020; Sutiknowati, 2016).

MATERIAL AND METHODS

Collection of Samples

Date palm used are fresh, brown colour were purchased from shop in Sidoarjo.

Date Collected Extract Preparation

Dates are taken from the flesh and crushed into powder using electric blender and sieven as fine powder. The powder was stored in sterile containers until its use.

Formulation of Media

Flour date palm was weighted as much as 1 gram, 2 grams, 4 grams, 6 grams, 8 grams, then dissolved in 100 mL of distilled water in an Erlenmeyer by adding 1.5 grams of bacterial agar and 0.5 grams of glucose. Heat until dissolved and adjust pH 7.4±2 at 25°C, then sterilize in an autoclave at 121°C for 15 minutes, then pour into a petridisk and cool at room temperature until solid (Thohari et al, 2019).

Table1. Composition of Flour Date Palm

Concentration	Composition
1 g	Flour date palm 1 g + 1,5 g bacterial agar + 0,5 g NaCl + 0,5 g glucose + 100 mL aquadest
2 g	Flour date palm 2 g + 1,5 g bacterial agar + 0,5 g NaCl + 0,5 g glucose + 100 mL aquadest
4 g	Flour date palm 4 g + 1,5 g bacterial agar + 0,5 g NaCl + 0,5 g glucose + 100 mL aquadest
6 g	Flour date palm 6 g + 1,5 g bacterial agar + 0,5 g NaCl + 0,5 g glucose + 100 mL aquadest
8 g	Flour date palm 8 g + 1,5 g bacterial agar + 0,5 g NaCl + 0,5 g glucose + 100 mL aquadest
Control positive	*Nutrient agar* instant (NA)

Control negative	Bacteriological agar

Preparation of *Nutrient Agar* (NA)
NA media was weighed as much as 1.36 grams then dissolved 68 mL of distilled water in an erlenmeyer. The media was heated until completely dissolved, then measured pH 7.4 ± 0.2 using pH paper then autoclaved for 15 minutes at 121°C, then poured into a petri dish and allowed to solidify at room temperature.

Counting of Bacteria
Bacterial cultures used for analysis were *Escherichia coli, Bacillus cereus*. These bacteria were diluted 10^{-5} and inoculated on the treatment medium by the spread plate method then incubated at 37°C for 24 hours. After incubation, the total number of bacteria was calculated using the TPC (*Total Plate Count*) method. Colony count by formula:

$$\text{Colony/g} = \sum \text{colony per plate} \times \frac{1}{\text{Dilution factor}}$$

The number of bacterial colonies counted was between 30 and 300 CFU/gr. If the number of colonies is more than 300 CFU/gr it is categorized as too many to count (TBUD). Thus, only plates that grew between 30-300 colonies were counted as the number of bacterial colonies inoculated (Azizah & Soesetyaningsih, 2020).

Data Analysis
Data analysis was carried out using a descriptive method, namely describing the results of the TPC. The analysis will be presented in tabular form to facilitate reading according to the Standard Plate Count (SPC).

RESULTS
Based on the results of the research conducted, the palm flour media produced different abundances of bacteria. The colony count, characteristic colony of *Escherichia coli* presented in Table 2, while *Bacillus subtilis* showed in Table 3. Both colony of *Escherichia coli* and *Bacullis cereus* increases with the weight of date flour.

Table 2. Calculation of the number of dates flour TPC colonies on *Escherichia coli*

Variation of Concentration Media (g)	Abundace Colony (total x 10^5CFU/g)	Characteristic Colony			
		Form	Elevation	Margin	Colour
1	15	Circular	Raised	Entire	White Opaque
2	24	Circular	Raised	Entire	White Opaque
4	36	Circular	Raised	Entire	White Opaque
6	42	Circular	Raised	Entire	White Opaque
8	54	Circular	Raised	Entire	White Opaque
Control Positive	71	Circular	Raised	Entire	White Glossy
Control Negative	-	-	-	-	-

Table 3. Calculation of the Number of Dates Flour TPC Colonies on *Bacillus cereus*

Variation of Concentration Media (g)	Abundace colony (total x 10^5CFU/g)	Characteristic colony			
		Form	Elevation	Margin	Colour
1	10	circular	Raised	entire	White opaque
2	21	circular	Raised	entire	White opaque
4	32	circular	Raised	entire	White opaque
6	40	circular	Raised	entire	White opaque
8	45	circular	Raised	entire	White opaque
Control positive	64	circular	Raised	entire	White glossy
Control negative	-	-	-	-	-

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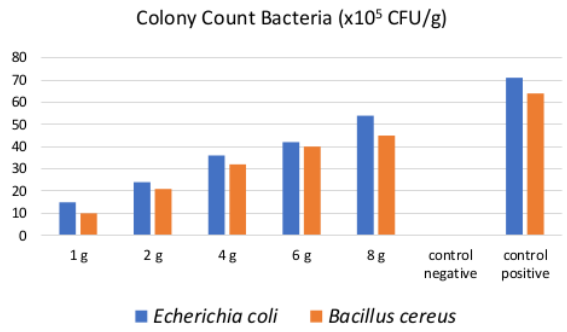

Figure 1. Colony count Bacteria

Table 2 shows that the highest colony abundance on NA media was 71 x 10^5 CFU/g, with variations in the concentration of date palm flour media 1 g, 2 g, 4 g, 6 g, 8 g at 15 x 10^5, 24 x 10^5, 36 x 10^5, 42 x 10^5, 54 x 10^5 while the negative control did not find colonies. Table 3 shows that the variation of the media concentration of 1 g, 2 g, 4 g, 6 g, 8 g is 10 x 10^5, 21 x 10^5, 32 x 10^5, 40 x 10^5, 45 x 10^5, positive control is 64 x 10^5 while the negative control did not grow colonies. Figure 1 showed that the most optimal bacterial growth was in NA media treatment, while the growth of *Escherichia coli* was better than the growth of *Bacillus cereus*.

DISCUSSION

Bacterial growth on NA media grows more optimally because NA media has been tested for bacterial growth media. *Nutrient agar* (NA) media is a common medium used to breed bacteria with high nutritional content consisting of meat extract, yeast extract, peptone. Meat extract acts as a source of carbon, nitrogen, oxygen, minerals, vitamins. Peptone is a protein source of carbon, nitrogen, oxygen and sulfide which are needed by bacteria to grow and develop. Bacteria will not grow if the nutrient content of the growth medium is lower. Based on the observation, bacterial colonies grew faster in NA media than in date palm flour media this is because NA media has been clinically tested for bacterial growth and date palm flour contains compounds that are still complex causes the growth of microorganisms to take longer to decompose the components into simpler ones so that they are easily absorbed by cells and used for cell synthesis. If the nutrient content and elements are adequate, bacterial growth is relatively fast and contrarily if the nutrients needed for bacterial growth are lacking or not abundant, the cells will adapt to the environment and take longer for the formation of enzymes to decompose the substrate (Gandjar, 2006). The addition of agar to this alternative media serves as a solidifier for culture and glucose as a carbon source. Bacteria are single-celled organisms that reproduce in a simple way. For growth it requires nutrients. Microorganisms require 10 macroelements (C, O, H, N, S, P, K, Ca, Mg, Fe). The first six components are needed to synthesize carbohydrates, lipids, proteins and nucleic acids and the other four serve as cations in cells. In addition, microorganisms also need microelements such as Mn, Zn, Co, Mo, Ni, Cu, microbes also need growth factors such as organic compounds (Basu et al., 2015).

In each concentration variation of *Escherichia coli* and *Bacillus cereus* there are differences in the number of growth colonies this is due to several factors, namely abiotic and biotic factors, the structure of the bacterial cell wall. Abiotic factors include nutrition, pH, temperature, osmotic pressure, light, humidity, oxygen, antimicrobial compounds. Biotic factors include associations or co-existence between microorganisms (Fardiaz 1993; Hadioetomo 1993; Jawetz 2014). The number of colonies growth of *Escherichia coli* and *Bacillus cereus* showed different results, the number of colonies of

Escherichia coli bacteria was more than the number of colonies of *Bacillus cereus.* this was due to differences in cell wall structure between *Escherichia coli* and *Bacillus cereus.* Bacteria *Escherichia coli* are Gram negative that have a more complex cell wall consisting of three layers, namely the outer layer in the form of lipoproteins, the middle layer in the form of polysacharides and the inner layer in the form of peptidoglycan, and has lipid content of about 11-12% so that Gram negative are more resistant to environmental changes caused by chemicals and the lysis of cell wall peptidoglycan. While *Bacillus cereus* is a Gram positive that has a simpler cell wall structure consisting of a layer of peptidoglycan, teichoic acid (polysacharide) and a little lipid so that the cell wall of Grampositive bacteria is more easily damaged if there are changes in the surrounding environment, without a cell wall bacteria cannot survive against outside influences and die quickly (Brock, 2014; Pelczar 2008; Jawetz 2014).

The growth medium and environment can also affect the growth of bacterial colonies. If the growth media and environment conditions are the same as the previous growth media and environment, bacteria will quickly adapt so that bacterial colonies will grow quickly, but if the media and environmental conditions are different from the previous media and environmental conditions, bacteria need time to adapt to synthesize enzymes that needed for metabolism (Brock, 2014).

The macroscopic morphological characteristics that grew on NA media and date palm flour media had different morphological characteristics. NA media was overgrown with date flour media. Based on the color characteristics of the colonies, it was shown that most of the colonies that grew were opaque white to glossy white. Based on the shape, surface and edge of the colony, *Escherichia coli* has a macroscopic morphology of circular, raised, entire, while Bacillus cereus has a macroscopic morphology of circular, flat, entire. Based on the description above date flour can be used as an alternative medium for bacterial growth.

CONCLUSION

Based on the results of the study, it can be concluded that *Escherichia coli* and *Bacillus cereus* can grow in date flour media with the number of *Escherichia coli* colonies more than the number of *Bacillus cereus* colonies that is is 54 x 10^5CFU/g on concentration media 8 gr. Dates flour media can be used as a cheap alternative culture medium.

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