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ISOLATION AND CHARACTERIZATION OF CELLULOLYTIC BACTERIA DURING NATURAL FERMENTATION OF SWEET ORANGE PEEL WASTE (*Citrus sinensis*)

ISOLASI DAN KARAKTERISASI BAKTERI SELULOLITIK SELAMA FERMENTASI ALAMI SAMPAH KULIT JERUK MANIS (Citrus sinensis)

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Abstract

Orange peel is one of organic waste which contains fibers, such as cellulose and hemicellulose utilized by cellulolytic microorganisms as growth media in the fermentation process. Cellulolytic microorganisms are widely used in many industries. This research will observe the profile of bacterial colonies, particularly cellulosic bacteria, during the fermentation of orange peels (*Citrus sinensis*). Fermentation was carried out during the research process; the bacteria were further isolated in Carboxymethyl Cellulose (CMC) media. The fermentation process was performed for 14 weeks where sampling on the first week was done every day for five days (H0–H4), while sampling from the 2nd to 14th weeks were conducted once a week (M2–M14). The isolation process was carried out in a Nutrient Agar medium with spreading method by calculating the Total Plate Count (TPC) of bacterial colonies and observing the macroscopic morphology of bacterial colonies. Bacterial counts are expressed in Colony Forming Units (CFU)/mL or viable count/mL. The identification of bacterial genus was based on the Bergey's Manual of Determinative Bacteriology. Bacterial isolation from the fermentation of sweet orange peel resulted in 20 isolates where 16 isolates were found to be cellulolytic bacteria through qualitative test in CMC agar plate. The hypothetic genus of 16 bacterial isolates were *Eubacterium, Cellulomonas, Microbacterium, Micrococcus, Planococcus, Pseudomonas, Azotobacter, Azomonas, Flavobacterium, Cytophaga*, and *Jonesia*. Isolate F15 (*Cytophaga* and *Azomonas*) was found to dominate the growth, while other isolates grew alternately with lesser frequency. Hypothetic genus of bacteria actively involved in the process were cellulolytic bacteria, allowing the liquid of fermentation products to be possibly used in the application.

Keywords: Cellulolytic bacteria; Fermentation; Orange peel

Abstrak

Kulit jeruk merupakan salah satu limbah organik yang mengandung serat seperti selulosa dan hemiselulosa yang dapat dimanfaatkan oleh mikroorganisme selulolitik sebagai media pertumbuhan dalam proses fermentasi. Mikroorganisme selulolitik telah digunakan di banyak industri. Penelitian ini mengamati profil koloni bakteri selama proses fermentasi kulit jeruk terutama bakteri selulotik. Selama proses penelitian dilakukan proses fermentasi, lalu bakteri diisolasi menggunakan media Carboxyl Methyl Callulose (CMC). Proses fermentasi dilakukan selama 14 minggu dengan rincian sampling pada Minggu ke-1 dilakukan setiap hari selama 5 hari (H0–H4), sedangkan minggu ke-2 hingga 14 dilakukan setiap seminggu sekali (M2–M14). Proses isolasi dilakukan dalam medium Nutrient Agar dengan teknik sebar dengan perhitungan koloni Total Plate Count (TPC) dan pengamatan morfologi koloni bakteri secara makroskopis. Hasil perhitungan bakteri dinyatakan dalam Colony Forming Units (CFU)/mL atau viabel count/mL. Pendugaan genus bakteri berdasarkan Bergey's Manual of Determinative Bacteriology. Hasil isolasi bakteri dari fermentasi kulit jeruk manis adalah 20 isolat yang 16 di antaranya merupakan bakteri selulolitik melalui uji kualitatif pada media plat CMC. Genus hipotetik bakteri dari 16 isolat adalah Eubacterium, Cellulomonas, Microbacterium, Micrococcus, Planococcus, Pseudomonas, Azotobacter, Azomonas, Flavobacterium, Cytophaga, dan Jonesia. Isolat F15 (Cytophaga dan Azomonas) mendominasi pertumbuhan, sedangkan isolat lain tumbuh berselang seling dengan frekuensi yang lebih kecil. Genus bakteri hipotetik yang terlibat aktif adalah bakteri selulolitik sehingga cairan hasil fermentasi dapat digunakan dalam aplikasi.

***Keywords**: Bakteri selulolitik; Fermentasi; Kulit jeruk*

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INTRODUCTION

Citrus fruit is a type of fruit widely consumed in the world. The increasing consumption of citrus fruits has an impact on the waste produced, namely orange peels. Orange peel contains 9.21% cellulose and 10.5% hemicellulose (Rivas et al., 2008). Cellulolytic microorganisms can utilize cellulose and hemicellulose as substrate to produce cellulase enzymes (Baharuddin et al., 2010) which play role in industrial applications, such as textiles, pulp, paper, and bioethanol (Balasaravanan et al., 2013). Moreover, the use of cellulolytic bacteria as a mixture of animal feed is useful for improving the quality of nutrients absorbed (Hernawati et al., 2010).

The utilization of organic waste through a 3-month natural fermentation developed by Dr. Rosukon Poompanvong (Bariyah, 2010) resulted in substance known as garbage enzyme. Rangaswamy et al. (2015) found bacterial isolate from rotten pomegranate, rotten sweet potato, and rotten potato, identified as *Gluconacetobacte*r sp. RV28, *Enterobacter* sp. RV11, and *Pseudomonas* sp., respectively. Garbage enzyme solution is also found to successfully reduce ammonia nitrogen and phosphates completely (Nazim, 2013). However, different fruits and vegetable wastes showed different enzyme and antimicrobial activity (Neupane & Khadka, 2019). Furthermore, another 3-month natural fermentation of a consortium of fruit peels (watermelon, orange, lime, blush, papaya, guava, banana, longan, mango, dragon fruit, and guava) in various combinations resulted in cellulase and lipase enzyme activity. Several combinations of fruit consortia, included watermelon and oranges, produced the highest cellulase and lipase enzyme activity (Sumarlin et al., 2013).

Another study considered that *Trichoderma* species was the most suitable candidate for cellulase production and utilization in the industry when compared to *Aspergillus* and *Humicola* species (Imran et al., 2016). The bacteria mostly isolated were mainly the member of the genera: *Erwinia, Xanthomonas, Pseudomonas*, and *Cytophaga*. The research of Maheshwari et al. (2018) clearly revealed a high level of diversity in the Lactic Acid Bacteria (LAB) microflora from fermented fruit mixtures. Arekemase et al. (2020) showed that lactic acid can be produced from mango, orange, and banana peels. Besides, the presence of bacterial or fungal colonies in orange, pineapple, banana and mixed fruit (pomelo, watermelon and melon) peels provided strong evidence that microbes produced cellulase in order to degrade carboxymethyl cellulose (CMC) (Chin et al., 2018).

According to Kusumaningati et al. (2013), fermentation of vegetable and fruit waste with the help of *Zigomomonas mobilis* produced ethanol when inoculum was added. However, without the addition of inoculum, ethanol is not produced. Isolation of cellulolytic bacteria found during the fermentation of orange peels is expected to accelerate the fermentation process, hence improving the efficiency of fermentation time besides producing the desired fermentation product. The natural fermentation process for 3 months has never been applied, especially to orange peel waste (*C. sinensis*) regarding the bacteria that play important role during the process. In this initial stage, the study focuses on cellulolytic bacteria as they are commonly found in organic materials and used in environmentally-friendly industrial activities. The objectives of this study/research include investigating the profile of bacterial colonies during the fermentation process of sweet orange peels and determining the potential of cellulolytic bacteria as they decompose organic matter from sweet orange peels (*C. sinensis*) during fermentation.

MATERIALS AND METHODS

The materials used in this study were citrus fruit peels belonging to the type of sweet orange (*C. sinensis*), palm sugar, Nutrient Agar (NA), CMC media, American Bacteriological Agar, MR-VP (Methyl Red-Voges Proskauer) media, OF (Hugh and Leifson's) media, Simmon's Citrate Agar media, and Triple Sugar Iron Agar (TSIA) media.

Fermentation

Orange peel waste were chopped, rinsed with 0.9% NaCl solution, put in a plastic storage container or fermentation tank, and added with palm sugar and water to a ratio of 3 kg orange peel:1 kg palm sugar:10 L water. This comparison is in accordance with the natural fermentation method

(Bariyah, 2010). Fermentation was carried out for 3 months and pH was measured every sampling time.

Fermentation was carried out during the research process; the bacteria were further isolated in Carboxymethyl Cellulose (CMC) media. The fermentation process was performed for 14 weeks where sampling on the first week was done every day for five days (H0–H4), while sampling from the 2nd to 14th weeks were conducted once a week (M2–M14). The isolation process was carried out in a Nutrient Agar medium with a spreading method by calculating the Total Plate Count (TPC) of bacterial colonies and observing the macroscopic morphology of bacterial colonies. Bacterial counts are expressed in Colony Forming Units (CFU)/mL or viable count/mL.

Isolation and Characterization of Bacteria

Approximately 1 mL of sample was taken from the fermenter to be further added to 9 mL of sterile physiological (0.9% NaCl) solution. Later, serial dilution was performed by diluting 1 mL of sample into 9 ml of sterile physiological (0.9% NaCl) solution. As much as 0.1 mL of the result of each dilution series was taken, spread on Nutrient Agar (NA) medium, and incubated at room temperature for 24 hours to grow.

Isolation of bacteria was applied to liquid sample and dilution was carried out by transferring 10^1 to 10^8 of fermentation liquid in the fermenter during the fermentation process, growing it on NA media (see the sampling method above), and incubating it for 24 hours at 37 °C. The growing bacterial colonies were observed and sub-cultured as stock culture, while total bacterial count was done on NA media using the Total Plate Count (TPC) method (Madigan et al., 2009).

Bacterial characterization was carried out by observing the colony characteristics of pure isolates (color, edges, surface, shape) (Cappuccino & Sherman, 2001), Gram and cell staining (Madigan et al., 2009). Moreover, biochemical testing included motility test, catalase test, MR-VP oxidase, indole test, citrate test, TSIA, gelatin test, and fermentation oxidation test, followed by bacterial genus identification based on Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

Screening for Cellulolytic Bacteria

Cellulolytic bacterial isolates were tested qualitatively by observing the clear zone produced from the degradation of cellulose. The stock cultures of bacterial isolates were sub-cultured and incubated at 37 °C for 24 hours prior to testing for their activity. Screening for cellulolytic bacteria was performed by using streak plate technique on CMC plate media to be further incubated at 37 °C for 48 hours. Cellulolytic activity testing was carried out using the Gram staining method. The colonies grown on the medium were added drops of Gram's Iodine for 3-5 minutes and observed for the presence or absence of a clear zone formed (Kasana et al., 2008).

RESULTS

Bacterial observation from the natural fermentation process of orange peels conducted for 14 weeks resulted in 20 isolates. Characteristics of 20 bacterial isolates are presented in Table 1. Colony morphology of 20 isolates showed that 9 isolates were in the form of circles with entire edges and convex surfaces. In term of cell characteristics, 12 isolates were observed to have cocci shape and 8 isolates had rod-shaped cells (Figure 1). The differences in morphological characters and microscopic observations were followed by test of the biochemical activity to determine the genus. Identification of the bacterial genus for each isolate was performed based on the similarity of characteristics according to Bergey's book of Manual Determinative Bacteriology 9th edition.

Screening for cellulolytic bacteria from fermentation of orange peels resulted in 16 isolates with ability to produce a clear zone, later classified as cellulolytic bacteria (Table 2). The growth of bacterial consortium for 14 weeks fluctuated over time, thus indicating a succession. Figure 2a and 2b show the fluctuation of bacterial growth during orange peel fermentation.

The initial pH during fermentation (Day-0) was 4.8, but there was a significant decrease to 3.58 on day-1. Started from the initial stage of fermentation until the 14th week, pH values ranged

from 3.1 to 4.8. However, decrease in pH value occurred from week 1 to 6, and from week 9 to 10 (Figure 3).

Table 1. Characteristics of bacterial isolates from fermentation of orange peels (*Citrus sinensis*)

Isolate code	Colony characteristics				Cell characteristics		Frequency of appearance (times)
	Shape	Edge	Surface	Color	Gram	Form	
F1	*Irregular*	*Entire*	*Raised*	Dull white	Positive	*Basil*	5
F2	*Circle*	*Entire*	*Convex*	Glossy white	Positive	*Coccus*	6
F3	*Circle*	*Entire*	*Convex*	Clear yellow	Positive	*Basil*	4
F4	*Circle*	*Entire*	*Convex*	Glossy white	Positive	*Coccus*	8
F5	*Circle*	*Entire*	*Convex*	Yellow	Positive	*Basil*	3
F6	*Circle*	*Entire*	*Raised*	Dull white	Positive	*Basil*	3
F7	*Circle*	*Entire*	*Convex*	Pink	Positive	*Basil*	1
F8	*Circle*	*Irregular*	*Flat*	Transparent white	Positive	*Basil*	2
F9	*Rhizoid*	*Entire*	*Raised*	White	Negative	*Coccus*	2
F10	*Circle*	*Entire*	*Convex*	Transparent white	Positive	*Basil*	1
F11	*Irregular*	*Entire*	*Flat*	White	Positive	*Coccus*	1
F12	*Irregular*	*Entire*	*Raised*	Dull white	Negative	*Cossus*	11
F13	*Irregular*	*Entire*	*Raised*	Dull white	Positive	*Coccus*	4
F14	*Circle*	*Entire*	*Flat*	White	Negative	*Coccus*	10
F15	*Circle*	*Entire*	*Convex*	Clear	Negative	*Coccus*	14
F16	*Irregular*	*Entire*	*Flat*	Dull white	Positive	*Coccus*	1
F17	*Irregular*	*Entire*	*Flat*	Dull white	Negative	*Coccus*	1
F18	*Circle*	*Entire*	*Convex*	Transparent white	Negative	*Coccus*	3
F19	*Irregular*	*Lobate*	*Raised*	Dull white	Positive	*Basil*	1
F20	*Irregular*	*Entire*	*Flat*	Dull white	Positive	*Coccus*	3

Note: *Rhizoid*= fibers; *circle*= round; *irregular*= irregular; *entire*= smooth; *undulate*= uneven; *lobate*= irregular; *flat*= flat; *raised*= arise; convex= convex

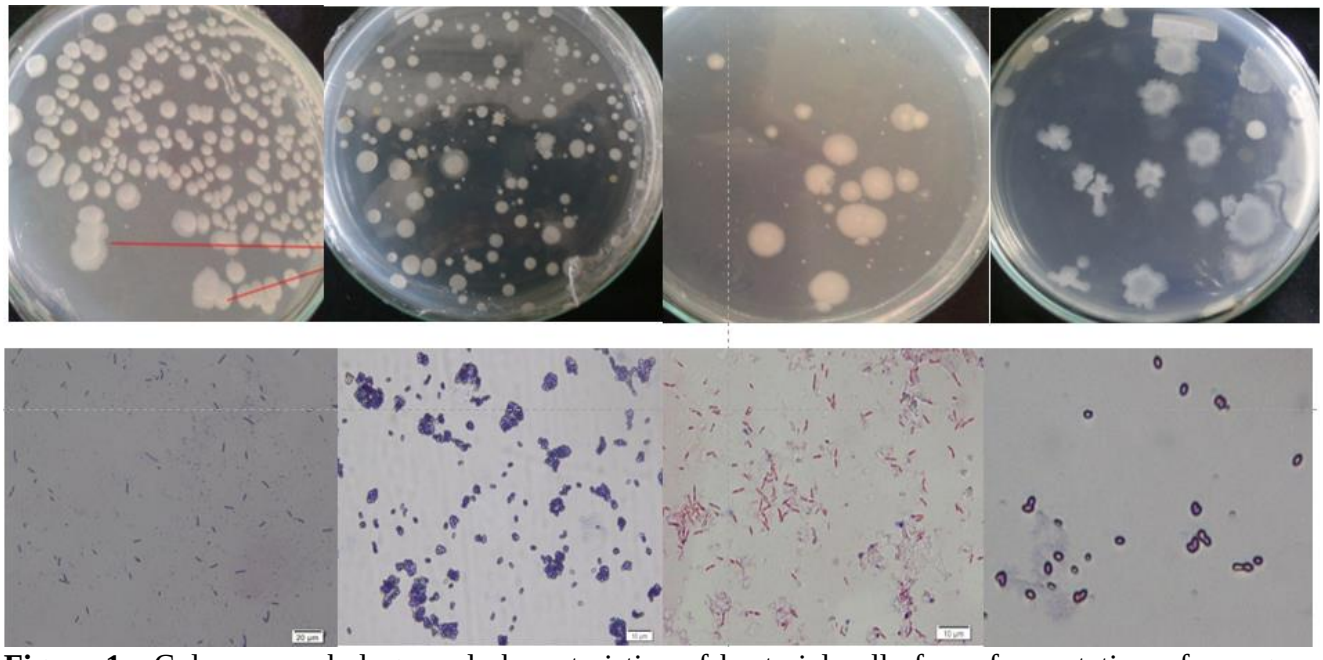

Figure 1. Colony morphology and characteristics of bacterial cells from fermentation of orange peels (*Citrus sinensis*)

Table 2. Result of screening test for cellulolytic bacterial isolates from fermentation of orange peels (*Citrus sinensis*)

Isolate code	Clear zone	Isolate code	Clear zone	Isolate code	Clear zone	Isolate code	Clear zone
F1	+	F6	+	F11	-	F16	+
F2	-	F7	-	F12	+	F17	+
F3	+	F8	-	F13	+	F18	+
F4	+	F9	+	F14	+	F19	+
F5	+	F10	+	F15	+	F20	+

Note: + (positive for cellulolytic producer); - (negative for cellulolytic producer)

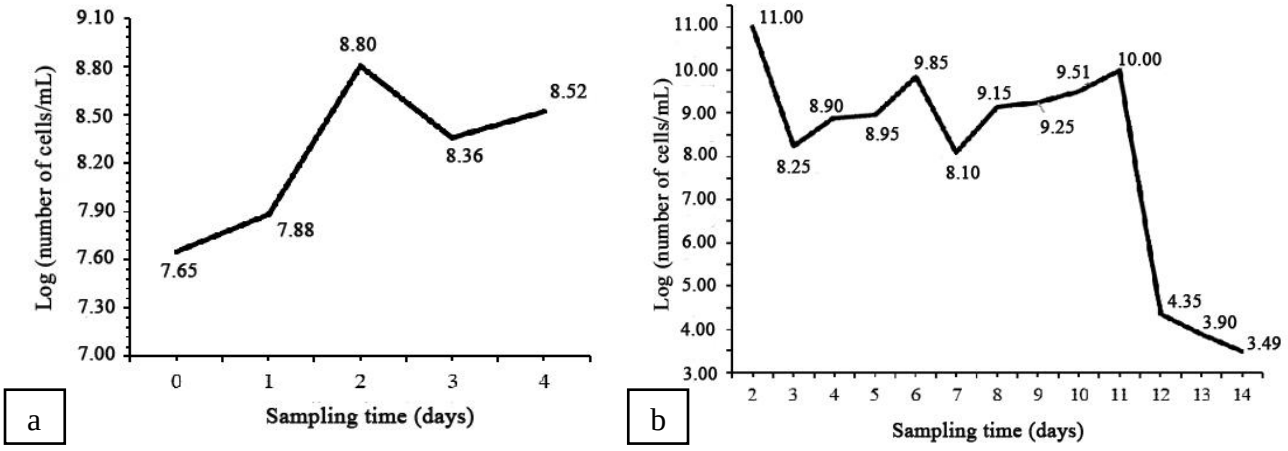

Figure 2. The pattern of bacterial growth during the fermentation process of orange peels (*Citrus sinensis*) during sampling at week 1 (a), and at week 2 to 14 (b)

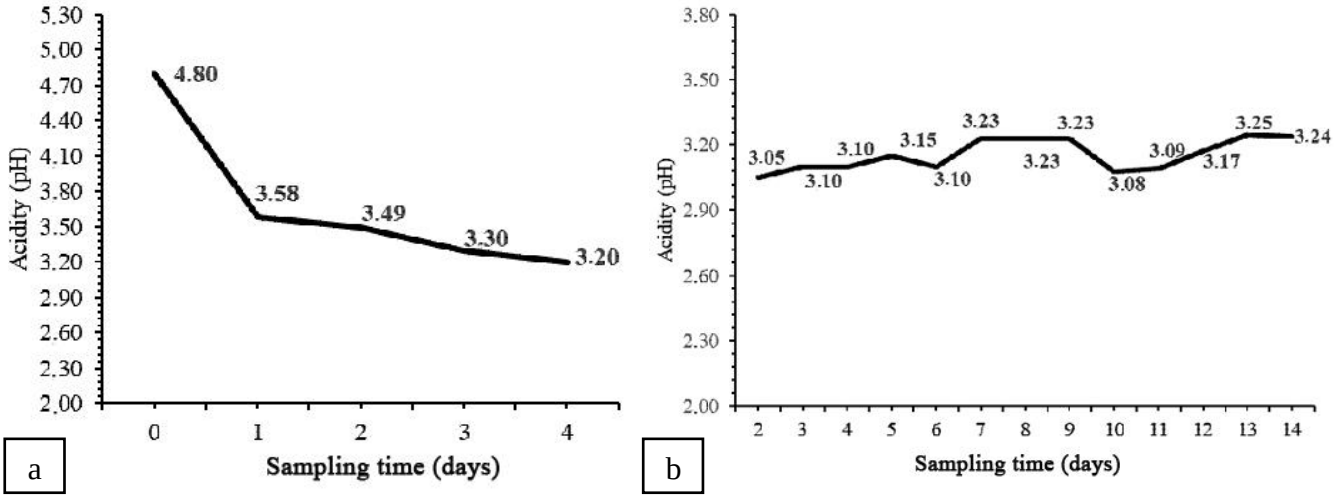

Figure 3. Changes in pH during the fermentation process of orange peels (*Citrus sinensis*), during sampling at week 1 (a), and at week 2 to 14 (b)

The profile of bacterial colonies was used to determine the number of bacteria that played role in the fermentation process of orange peels. A total of 20 isolates were successfully obtained. Isolation in week 1 (Day 0 to 4) resulted in 15 isolates, those were F1-F15 (Figure 4a). Other isolates were also obtained, namely isolate F16 and F17 obtained in week 2, isolate F18 found in week 3 (Figure 4b), isolate F19 observed in week 8 (Figure 4c) and isolate F20 obtained in week 12 (Figure 4c). The appearance of various bacterial isolates is expectedly caused by the time required by bacteria to adapt to the new environment.

Biochemical activity test applied to 16 isolates obtained from the fermentation of orange peels resulted in 11 different genera, including *Eubacterium, Cellulomonas, Micrococcus, Pseudomonas,*

Azotobacter, *Flavobacterium*, *Cytophaga*, *Microbacterium*, *Azomonas*, *Planococcus*, and *Jonesia* (Table 3).

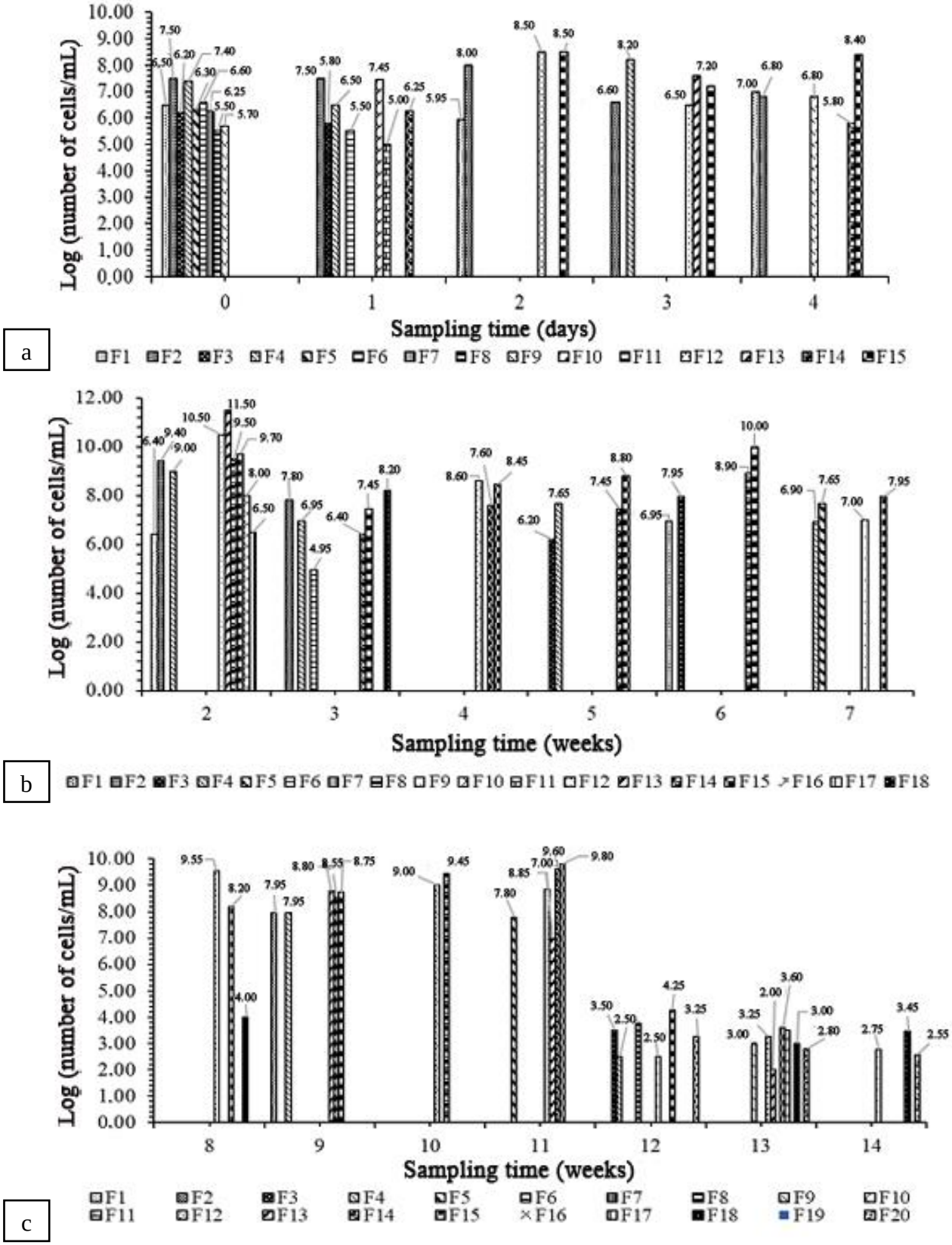

Figure 4. Profile of the presence of bacterial isolates at week 1 (a), week 2 to 8 (b), and week 8 to 14 (c) during the fermentation process of orange peels (*Citrus sinensis*). F= isolate code

Table 3. The results of biochemical activity testing of cellulolytic bacterial isolates during the fermentation of orange peels (C. *sinensis*)

Isolate/ properties	Gram	Mot	Cat	Ox	MR	VP	Indole	Citrate	TSIA				Gel	OF	Identification of Bacterial Genus (Holt et al., 1994)
									Glu	Gas	H_2S	Lac			
F1	+	+	-	-	+	-	-	-	Y	-	-	R	-	-	*Eubacterium* and *Lactobacillus*
F3	+	+	+	-	-	-	+	+	Y	-	-	R	+	+	*Cellulomonas* and *Microbacterium*
F4	+	-	+	+	-	-	-	+	Y	+	-	Y	-	+	*Micrococcus* and *Trichococcus*
F5	+	-	+	-	-	-	+	-	R	-	-	R	+	+	*Micrococcus, Planococcus,* and *Trichococcus*
F6	-	-	+	-	-	-	-	-	Y	-	-	R	-	+	*Acinetobacter, Pseudomonas,* and *Aminobacter*
F9	-	-	+	+	-	-	-	-	R	-	-	Y	-	+	*Azotobacter* and *Xanthobacter*
F10	-	-	+	+	+	-	-	-	Y	-	-	R	+	+	*Flavobacterium* and *Pseudomonas*
F12	-	+	+	-	+	-	-	-	Y	-	-	R	+	+	*Azotobacter* and *Cytophaga*
F13	+	-	+	+	+	-	-	-	Y	+	-	R	+	+	*Micrococcus* and *Microbacterium*
F14	-	-	+	+	-	-	+	+	Y	-	-	R	-	+	*Azotobacter* and *Xanthobacter*
F15	-	+	+	-	+	-	+	+	Y	+	-	Y	+	+	*Cytophaga* and *Azomonas*
F16	+	-	+	-	-	-	-	-	Y	-	-	R	+	+	*Micrococcus, Planococcus,* and *Trichococcus*
F17	-	-	+	-	-	-	+	-	Y	-	-	R	+	+	*Azotobacter* and *Cytophaga*
F18	+	+	+	-	-	-	-	-	Y	-	-	R	+	+	*Micrococcus, Planococcus,* and *Trichococcus*
F19	+	-	+	-	-	-	-	-	Y	-	-	R	-	+	*Jonesia* and *Corynebacterium*
F20	+	-	+	-	-	-	-	-	Y	-	-	R	+	+	*Cellulomonas* and *Microbacterium*

Note: + (positive); - (negative); mot (motility); cat (catalase); ox (oxidase); MR (*Methyl Red*); VP: *Voges Proskauer*; glu (glucose); lac (lactose); gel (gelatin); OF (oxidation fermentation: Y= yellow; R = red); * positive identification result of bacterial genus is written in bold, TSIA: *Triple Sugar Iron Agar*

DISCUSSION

The observation result of colony morphology of 20 isolates obtained from the fermentation of orange peels showed that 9 isolates were in the form of circles with entire edges and convex surfaces, while cell characteristics indicated 12 isolates with cocci shape and 8 isolates had rod-shaped cells. The results of Gram staining showed that 14 isolates were Gram positive, while 6 isolates were Gram negative (Figure 1).

Each bacterial isolate did not always appear for 18 times of sampling time. Several bacterial isolates appeared more than once during the sampling time, for example isolates F15, F14, and F12 with appearance frequency of more than 10 times out of 18 times of sampling time (Table 1). The isolates found at different sampling times were affected by the process of adaptation and competition between consortium members, causing different growth rate between isolates. Available nutrient sources will have impact on varied bacterial growth, whereas an uneven distribution process and the influence of consortium members can create competition in bacterial growth (Madigan et al., 2009).

The positive results of cellulolytic bacteria were indicated by the formation of a clear zone on the selection media, as a sign of microbial activity in degrading cellulose (Kasana et al., 2008). The test results showed that most isolates were cellulolytic bacteria, presuming that the composition of the ingredients used could affect the cellulose enzyme activity of microorganisms in the fermentation process of orange peels (*C. sinensis*). During the fermentation process, cellulolytic bacteria were found in the decomposition of organic matter obtained from orange peels; this may be due to cellulose and hemicellulose content in orange peels, which can be a source of energy by cellulolytic bacteria (Hatami et al., 2008). Andritsou et al. (2018) isolated pectinase and cellulase (cellulolytic) from solid-state fermentation of orange peels, while Adeleke at al. (2012) used *Penicillium atrovenetum, Aspergillus flavus,* and *Aspergillus oryzae*. Citrus peels (lemon, mandarin, orange and grapefruit) were also used for bacterial cellulose production (Güzel & Akpinar, 2019).

During the fermentation of orange peels, the measurement results showed fluctuations at week 1 (Day-0 to D-2), week 1 to 2, week 3 to 6, and week 7 to 14 (Figure 2). The fluctuated growth of cell concentrations indicated a succession of the bacterial consortium. According to Rui et al. (2009), the initial stage of succession was marked by a high increase in cell numbers followed by a sharp decrease in cell numbers at the next step. The activity of bacteria influences the increase in growth in fermentation by utilizing the substrate derived from orange peel. Microorganisms can use the substrate from grape skins to hydrolyze these compounds to produce ethanol and other fermentation end products (Wilkins et al., 2007).

Figure 2 shows the increase in bacterial cell concentration at week 1 to 2, week 4 to 4, and week 8 to 11. The increase in cell concentration indicated a growth process for members of the bacterial consortium. The presence of a high bacterial growth rate at the beginning of the fermentation may be due to the activity of bacteria in utilizing abundant glucose and sucrose as an energy source. The increase of bacteria growth occurred at week 4 to 6 and week 8 to 11, allegedly members of the bacterial consortium began to utilize co-substrate from orange peels by synthesizing cellulose enzymes.

During the fermentation of orange peels, the number of bacteria cells decreased at week 3, 7, 12, and 14, as found in Figure 2. The decrease in cell concentration is possibly influenced by the accumulation of compound that is toxic to cells and the deamination process of microorganism activity, hence resulting in a decrease in growth rate and limited sources of nutrients. In fact, limited sources of nutrition and competition will make it difficult for microorganisms to survive, causing a decrease in the number of cells (Llorens et al., 2010). Hawashi et al. (2018) found that optimization of the fermentation time and bacteria cell number in the starter culture, also the logarithmic phase of the *L. plantarum* growth curve was obtained from 6 to 16 h, followed by a decrease in growth as marked by the decreasing number of bacterial cells.

The value of pH was measured to determine the activity of bacteria in producing metabolites, such as organic acids (Figure 3). Prior to fermentation, pH measurement resulted in a value of 4.8. However, acidity in orange peels can influence the pH value that is classified as acidic, namely 3.42

(Martin et al., 2010). During fermentation, pH ranged from 3.1–4.8. The fluctuation in pH value is influenced by the activity of microorganisms in utilizing nutritional sources.

The decrease in pH is affected by the activity of microorganisms in decomposing organic matter and producing organic acids. The production of these organic acids causes the pH to decrease and the resulting organic acids are absorbed by bacteria, thus increasing bacterial growth. The results of the activity of microorganisms in the form of organic acids can be succinic acid, acetic acid, ethanol, and lactic acid (Cristina et al., 2008). The increase in pH value can also be affected by the deamination process. Deamination is a process of catalyzing the removal of amino groups from amino acids and other molecules containing NH, this process can cause the growth of microorganisms to be stunted and an increase in pH (Prescott et al., 2005).

The appearance of various bacterial isolates is expected due to the adaptation time required by bacteria (Table 1). The difference in appearance frequency of each isolate is possibly caused by the presence of a group of bacteria that requires a slower growth time in the adaptation process (Davis et al., 2005).

The dominant isolates from 18 sampling times, namely F15 (Red), F14 (Green), and F12 (Yellow) had a higher frequency of appearance and were relatively stable compared to the other isolates since the beginning of the sampling process (Figure 3a, 3b, 3c, and Table 1). Less number of bacteria found during sampling is suspected due to competition in nutritional needs by colonies that have the ability to grow quickly (Davis et al., 2005). Appearance frequency of each bacterial isolate during the fermentation process was relatively more stable as indicated by the appearance of several dominant bacterial isolates during the sampling time. The dominance of the isolates' presence can be influenced by the source of nutrition and the occurrence of competition during the fermentation process. Limited sources of nutrition and the existence of competition will make other microorganisms face difficulty to survive, resulting in a decreasing number of cells (Llorens et al., 2010).

Identification of the bacterial genus for each isolate was done by observing the similarity of characteristics according to Bergey's Book of Manual Determinative Bacteriology 9th edition. The results obtained were based on a combination of Matching Bacteria from Biochemical Activity Testing and Morphological Identification Observations, thus resulting in two genera for one isolate (Table 3). For example, the test result showed that isolate F4 had the same characteristics as *Micrococcus* and *Trichococcus* (Table 3). Moreover, 12 out of 14 biochemical characteristics of isolate F4 were similar to that of *Micrococcus*. Therefore, the result identified that the isolate belonged to the genus *Micrococcus.* Furthermore, based on the ability of this genus to decompose sawdust, the isolate is expected to be cellulolytic bacteria (Lennox et al., 2010).

CONCLUSION AND SUGGESTIONS

Based on the results of this study, it is concluded that 16 of total 20 isolates successfully obtained from the fermentation process of orange peels were identified as cellulolytic bacteria. Moreover, fermented water is potentially used in various applications related to the role of cellulolytic bacteria.

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