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Isolation And Characteristics Of Lactic Acid Bacteria In Feces Of Jember Local Mongoose

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Abstract: Drinking a cup of coffee is not only a matter of taste but also a part of Indonesian's daily life. Coffee shops which have proliferated so far proves this lifestyle. One of the best selling coffee bean products is mongoose coffee. It is a product of coffee beans which have been swallowed by mongoose and through its digestive tract. In the digestive tract, beans are being fermented by microbes (lactic acid bacteria or LAB). This fermentation process contributes to the unique taste and aroma of the coffee. The taste and aroma are very appealing to coffee lovers, resulting in the rise of demands in both local and international markets in every year. However, this leads to the escalation of mongoose hunting, threatening the population of mongoose (Paradoxurus hermaphroditus). Moreover, its limited digesting capacity also hinders the production of mongoose coffee. The present study offers an alternative to produce mongoose coffee by optimizing in-vitro fermentation which simulates the natural process of fermentation inside mongoose's digestive organs. This in-vitro fermentation uses bacteria isolate (LAB) from mongoose's feces collected from the local society of Jember. The study, therefore, aimed to: 1) isolate LAB from mongoose's feces, and 2) identify the characteristics of collected LAB. Based on the result of data analysis, it was concluded that 5 LAB isolates from local mongoose's feces were identified, namely: LAB-1 is Lactobacillus plantarum, LAB-2 is Lactobacillus brevis, LAB-3 is Leuconostoc paramesenteroides, LAB-4 is Leuconostoc mesenteroides, and LAB-5 is Streptococcus faecium.

Keywords: bacteria, lactic acid bacteria, mongoose (Paradoxurus hermaphroditus)

INTRODUCTION

Mongoose coffee is a product of coffee beans which have been swallowed by mongoose and through its digestive tract. Mongoose (Paradoxurus hermaphrodites) is a mammal that is within the weasel and mongoose (Viverridae). It is a nocturnal carnivore type of mammal. Despite its nature as a carnivore, mongoose also eats ripe fruits, and one of them is fully ripe coffee beans [1].

A coffee bean is coated by hard skin that cannot be digested by mongoose, therefore it is let out in one piece along with its feces. During the digestive process, the coffee has been through a fermentation process for ±12 hours within mongoose's digestive tract. While the beans that cannot be digested are excreted in the feces during the excretion process [2].

The fermentation process that occurs inside mongoose contributes to produce a unique taste and aroma [3]. Such taste and aroma are of the attractive value to coffee lovers, resulting in the rise of the product demands in both local and international markets in every year [4].

The year-to-year increase of demands happens in several countries, such as Germany, Japan, the United States, and some European countries. The continuous increase of demands, somehow, complicates the mongoose coffee producers [5]. The increase is also caused by the selling price of Indonesia's mongoose coffee is set at 600 Canada dolar per pon, and considered as the rarest and most expensive drink or coffee in the world [6] while [7] stated that the international price has reached $100 to $600.

As the demands keep increasing, the mongoose hunting is also in a rise; threathening the population of mongoose in Indonesia [8] [9]. Besides, as the mongoose gets rarer and its digestive capacity is limited, the production is also limited. One of the alternative to optimize the production of mongoose coffee is to use in-vitro fermentation by manipulating microbes (particularly lactic acid bacteria) to replicate the process of natural fermentation which occurs inside the mongoose digestive organs. The lactic acid bacteria is functioned as a culture starter for fermentation in food industry [10].

METHOD

The present study was conducted from January to June 2020. This laboratory research took place in Microbiology Laboratory, the Basic Laboratory Unit of Universitas Muhammadiyah Jember, Jl. Karimata 49 Jember 68121.

The instruments to be used in this pre-research include alumunium foil, autoclave, biosafety cabinet, bunsen, petri dish, erlenmeyer, beaker glass, object glass and glass covers, measuring glass, hot plate, incubator, wrapping paper, oxidase test paper, fridge, magnetic stirrer, microscope, ose, drop pipette, and other tools used in the microbiology lab.

Meanwhile, the materials used for the purpose of the research are alcohol, aquades, congo red, glucose, hydrogen peroxide liquid (H2O2), lugol or iodine, coffee beans in mongoose feces (P. hermaphroditus) obtained from coffee farmers in Jember, crystal violet, CMC (Carboxy Methyl Cellulose) media, Natrium clorida (NaCl), Nutrient Broth (NB), gelatin media, Simmons Citrat Agar (SCA) media, Sulfit Indol Motility (SIM) media, TSIA (Triple Sugar Iron Agar) media, liquid paraffin, congo red reagent, Kovac's reagent, OF (Oxidative-Fermentative), and safranin.

A. Procedure

1) Bacteria Isolation from Mongoose Faeces

The process of bacteria isolation started from collecting 1 g of mongoose feces which was put into a test tube containing 9 ml of sterile aquades. This dilution process was done by taking 1 ml suspension of culture bacteria into the test tube (10-1) that contained yang berisi 9 ml of sterile aquades. The following process was to take 1 ml of diluted 10-1 and put it into the tube 10-2, and the process was repeated until 10-5. Afterwards, each suspense was taken as much as 1 ml from the test tube and put into the petri dish. The media used to grow the cellulolytic bacteria was the CMC media by applying the pour plate method.

2) Screening and Purifying Bacteria Isolates

Bacteria screening was done by observing the formation of clear zone on the CMC media. The growing bacteria which formed a clear zone on the media was examined to identify its morphological characteristics and then purified by applying the scratch method on the petri dish. Single bacteria isolates on CMC were grown using the scratch method in order to form a circle of bacteria with a diameter less than 1 cm on the petri dish, and then incubated for 24 hours under the temperature of 37oC. The growing bacteria isolates were given drops of congo red reagent 0,1% until covering the whole media surface for 1 minute. It was then washed using NaCl 1%. The positive result was indicated by the successful formation of clear zone in the surrounding of the bacteria colony.

3) Characteristics of Cellulolytic Bacteria

Bacteria isolates which are possible for the cellulolytic bacteria were characterized following the result of observed characteristics of colony morphology, cells, and biochemistry test [11] [12].

4) Bacteria Identification

Identifying proteolytic and cellulytic bacteria of mongoose feces was done by observing the common characteristics of the bacteria which have been characterized using the book of determination key from Bergey's Manual of Determinative Bacteriology.

5) Examination Parameter and Data Analysis of Bacteria Isolation

The parameter being examined in the present study is the bacteria colony which forms the clear zone, and which bacteria isolates characteristics containing examination on morphological characteristics of colony and cell, and physiological characteristics as seen from the biochemistry test. The gathered data was analyzed descriptively and presented in tables and figures.

RESULT AND DISCUSSION

The bacteria isolation which was assumed to be LAB resulted in 5 isolates. Afterwards, the isolates assumed to be LAB were identified in accordance with LAB characteristics.

Table 1. Characteristics of isolates, results from tests of cell shape, gram coloring, CO2, and catalase

No.	Isolate	Cell Shape	Gram	CO_2 Test	Catalase Test
1	LAB-1	coccus	+	-	-
2	LAB-2	Coccus	+	-	-
3	LAB-3	Coccus	+	-	-
4	LAB-4	Coccus	+	-	-
5	LAB-5	Coccus	+	-	-

Table 2. Characteristics of LAB isolates for growth in different temperatures

No.	Isolate	Growth in Temperature		
		15°C	**37°C**	**45°C**
1	LAB-1	+	++ +	+
2	LAB -2	+	+ + +	+
3	LAB -3	+	+	+ +
4	LAB -4	+	+ +	+ + +
5	LAB -5	_	+	+ + +

Table 3. Characteristics of LAB isolates by Acid Production Test (Litmus Milk) and Blue Litmus

No	Isolate	Growth	Precipitate	Result
1	LAB-1	Separation/clumping of milk, upper red, lower white	+ +	+ + +
2	LAB -2	Separation/clumping of milk, upper white, lower blue	+ + +	+
3	LAB -3	Separation/clumping of milk, upper white, lower blue	+ +	+
4	LAB -4	Separation/clumping of milk, upper red, lower white	+ + +	+ + +
5	LAB -5	Separation/clumping of milk, upper white, lower red	+ + +	+ +

Table 4. Characteristics of LAB isolates by Acid Production Test (Litmus Milk) and Red Litmus

No	Isolate	Growth	Precipitate	Result
1	LAB-1	Separation/clumping of milk, upper red, lower white	+ + +	+ + +
2	LAB -2	Separation/clumping of milk, upper white, lower blue	+ +	+ + +
3	LAB -3	Separation/clumping of milk, upper white, lower blue	+ +	+ + +
4	LAB -4	Separation/clumping of milk, upper red, lower white	+	+ +
5	LAB -5	Separation/clumping of milk, upper white, lower red	+ + +	+

Table 5. Characteristics of LAB isolates by Growth Test on Salt Concentration Variety

No	Isolate	Salt Concentration		
		0 %	**4 %**	**6,5 %**
1	LAB-1	**+ +**	+ + +	+ + +
2	LAB -2	**+ +**	+ + +	+ + +
3	LAB -3	**+ +**	+ + +	+ + +
4	LAB -4	**+ + +**	+ + +	+ +
5	LAB -5	**+ + +**	+ + +	+ +

Based on the results of isolation and characteristics test, 5 LAB isolates are obtained, namely: LAB-1 bacteria that is *Lactobacillus plantarum,* LAB-2 is *Lactobacillus brevis,* LAB-3 is *Leuconostoc paramesenteroides,* LAB-4 is *Leuconostoc mesenteroides,* and LAB-5 is *Streptococcus faecium*.

The finding of this study is confirmed by that of [13] stating that proteolytic bacteria in mongoose feces is *Bacillus* genus and *Proteus* genus. Meanwhile, [14] have isolated 3 bacteria isolates of Lactobacillus genus with the following species: *Lactobacillus plantarum, Lactobacillus fermentum,* and *Lactobacillus Jensenii*. Another previous study has identified *Lactobacillus sakei*, *Lactobacillus curvatus*, and *Lactobacillus plantarum* [15]. Generally, lactic acid bacteria involved in the fermentation are of the following species: *Lactobacillus casei, Lactobacillus coryniformis, Lactobacillus curvatus, Lactobacillus plantarum, Lactobacillus plantarum, Lactobacillus plantivum, Lactobacillus salivarius, Lactobacillus feractus, Lactococcus lactis, Pediococcus acidilactici, Pediococcus parvulus, Pediococcus pentosaceus* and those from Leuconostoc and Weissella genera [16].

LAB is commonly used as a culture starter in a fermentation and some of them ar also parts of the natural component of intestinal microflora [17]. Furthermore, they stated that the primary LAB are *Lactobacilli* and *Bifidobacteria,* as they are the most commonly used potential probiotic to improve health because they have several therapeutic functions. Besides, BAL can enhance aroma and flavor [18]

CONCLUSION

Based on the result and data analysis, the study is concluded in the following points: 1) bacteria which are within LAB of local mongoose feces of Jember include five LAB isolates, such as: LAB-1 is *Lactobacillus plantarum,* LAB-2 is *Lactobacillus brevis,* LAB-3 is *Leuconostoc paramesenteroides*, LAB-4 is *Leuconostoc mesenteroides*, and LAB-5 is *Streptococcus faecium*, and 2) the quality of coffee as examined from its taste in in-vitro fermentation results in two isolates with tastes closest to mongoose coffee, namely LAB-1 (*Lactobacillus plantarum*) and LAB-4 (*Leuconostoc mesenteroides*).

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