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Isolation and identification of *Lactobacillus* sp. bacteria in asian palm civet (*Paradoxurus hermaphroditus*) feces

Dinda Jelita Jauharah, Sri Chusniati*, Mohammad Anam Al Arif, Wiwiek Tyasningsih, Suryanie Sarudji, Agnes Theresia and Soelih Estoepangestie

Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

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ABSTRACT

The purpose of this research was to determine the presence of *Lactobacillus* sp. bacteria in Asian palm civet (*Paradoxurus hermaphroditus*) feces. This study totally used four samples of civet feces that obtained from civet farms at Cikole Lawang, Malang. The bacteriological isolation and identification of *Lactobacillus* sp. was determined by inoculation on pre-enrichment using de Man Rogosa Sharpe (MRS) *broth* and enrichment media using MRS Agar, followed by biochemically in Gram stain, Native isolate, Catalase test, Sulfide Indol Motility (SIM), Triple Sugar Iron Agar (TSIA), Methyl Red Voges Proskauer (MRVP), and Sugar fermentation test. Gram staining used to distinguish and classify bacterial species into two groups which is gram positive or gram negative and the result said that the bacteria has rods shape and including into Gram positive group. MRVP, TSIA, and sugar fermentation test are used to know the characteristic and fermentation ability of the bacteria. The results of this study showed that *Lactobacillus* sp. detected in all samples but in each samples has a different sugar fermentation abilities, one of the four samples has a motile character. Lactobacillus bacteria that grows may have different species.

Key words : *Viverridae, Lactobacillus, Agar*

Introduction

Pandan civet (*Paradoxurus hermaphroditus*) has a characteristic pandan leaf odor that can be smelled up to a radius of ± 20 meters, including members of the order carnivorous, but can also consume fruits, so it tends to be called frugivorous animals (Jothish, 2011). One of the advantages of mongoose pandanus in consuming coffee fruit is the ability to choose coffee fruit that has an optimum level of maturity, red and fresh by using the power of smell. Pandan Civet has the ability to choose coffee overnight able to consume 0.5 kg of ripe coffee. Coffee fruit consumed by mongoose will experience a relatively short digestion process, so that the flesh of the fruit is digested while the seeds will be removed with feces, because the coffee beans are protected by hard skin so it cannot be digested properly in the digestive tract of mongoose (Zahiroh, 2013). In the pulp of coffee contains 80% pectin and 20% sugar. Pectin has the ability to form a gel so it is very important in the process of making various food products such as jam, jelly, fruit preparation for yogurt, fruit juices and other products (Haryono *et al.*, 2012). Sugar is a substrate for microorganisms that can provide additional nutrients for lactic acid bacteria for metabolism and cell growth with the availability of optimal nutrition, then the activity of lactic acid bacteria will increase, causing the amount of acid produced by metabolism also increases (Maryana, 2014).

Lactobacillus sp. acts as a normal microflora in the digestive system of Luwak which has enzymes with activities to hydrolyze bile salt (bile salt hydrolase/ BSH), able to change the physical-chemical possessed by bile salts, so it is not toxic to *Lactobacillus* sp. Its function is to maintain the acid balance and alkaline so that the pH in the colon is constant. It has been tested to reduce pain and bloating. *Lactobacillus* sp. in vivo can reduce certain gastrointestinal symptoms and can increase the diversity of good bacteria in the large intestine (IGEM, 2009).

This research was conducted to determine the presence of *Lactobacillus* sp. in the pandan Luwak faeces (*Paradoxurus hermaphroditus*). *Lactobacillus* sp. Bacteria. obtained from pong mongoose stools as an alternative probiotics without the need for mongoose digestive tract surgery given the instability of the mongoose population in Indonesia which can then be used for fermentation of coffee beans outside the digestive system of mongoose pandan (*in vitro*).

Materials and Methods

This research was conducted at the Bacteriology and Mycology Laboratory of the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Airlangga University. The study was conducted from April to May 2019.

Pandan civet faecal samples, aquadest, violet crystal, lugol, alcohol, safranin, MRS agar and 'Merck' broth, SIM (Sulfide Indol Motility) media, TSIA (Triple Sugar Iron Agar) media, MR-VP (Methyl Red Voges media) Proskauer), a solution of confectionery

The tools used in this study include test tubes, test tube racks, flat pedestal glasses, microscopes, cotton, lighters, bunsen fires, pipettes, petri dishes, incubators, autoclaves, vortices, cool boxes, ice packs, erlenmeyers, aluminum foil, plastic wrap, loop loop, loop needle.

The data obtained were then displayed descriptively to illustrate the morphology and biochemical properties of the *Lactobacillus* sp. in the pandan Luwak faeces (Al-Arif, 2018).

Results and Discussion

In the results of the streak on the MRS media there is a milky white colony formation, so gram staining is done to determine the bacterial morphology microscopically from the bacterial colony. Microscopic results of gram staining test on the four samples showed that the bacteria were gram-positive and shaped as a purple stem, according to (Kavitha *et al.*, 2016). So that further biochemical tests are carried out to prove the characteristics of *Lactobacillus* sp. (Table 1).

Based on the results of biochemical tests conducted on the catalase test stated negative due to the absence of foam or foam when dripped with H_2O_2 solution, this is due to *Lactobacillus* sp. does not pro-

Table 1. Bacteria Identification Results of *Lactobacillus* sp. from the Pandan Luwak Stool

No	Test	Sample 1	Sample 2	Sample 3	Sample 4
1	Gram Staining	+	+	+	+
2	Catalase	-	-	-	-
3	SIM:				
	Indol	-	-	-	-
	Motility	—	—	—	—
4	Native	-	-	-	+
5	TSIA:				
	Oblique/straighten	Alkali/alkali	Alkali/alkali	Alkali/alkali	Alkali/alkali
	Gas	-	-	-	
	H_2S	-	-	-	-
6	MR/VP	±	±	±	±
7	Sugar test:				
	Glukose	-	+	+	+
	Sucrose	-	+	+	+
	Lactose	-	+	+	-
	Maltose	+	+	+	+
	Mannitol	+	-	+	-

duce catalase enzymes so the bacteria cannot break down hydrogen peroxide (H_2O_2) to H_2O + O_2 (Cahyono, 2007).

The results of SIM tests on all four samples showed the presence of bacterial cultures in the ose puncture area but the absence of inverted or foggy cypress formation that indicated the absence of motility in the bacteria. Then to determine the presence of indole dripped by kovac reagents there was no red ring change, which means that indole was declared negative (Islam *et al.,* 2016), and there was no black area indicating that H_2S was not produced by bacterial isolates (Chakraborty and Bhowal, 2015). In the SIM test results it is stated that there is no inverted or foggy cypress formation, but there are only bacterial cultures in the puncture ose area, so that the preparation of native preparations is made to prove the movement of bacteria (motile) in all samples. In all four samples, only sample no. 4 that there is movement (motile), while the sample no. 1, 2, and 3 there is no movement (not motile), *Lactobacillus* sp. most are not moving (not motile), but a small portion of the family of bacteria has a peritrichous flagella to move (Hammes and Hertel, 2009).

The TSIA test states the ability of bacteria to ferment three types of sugars, namely glucose, sucrose, and lactose. In the test results it is stated that sample no. 2, 3, and 4 are able to ferment the three sugars by changing the color of red to yellow on the TSIA media that has been stuck with Lactobacillus sp. which means the change of base to acid. In sample no. 1 there is no change in color which is still red which means the bacteria is unable to ferment these sugars, this is because each species of the genus Lactobacillus has a different ability to ferment sugars (Tambunan, 2016).

MR test to detect the presence of mixed acid fermentation and VP test to identify the formation of acetyl methyl carbinol. The results obtained by MR media which have been inoculated by bacteria from the four samples after the MR reagent drops showed a change in color to red which means MR was positive, while the VP test was no change in color, which was yellow which was stated as negative VP which meant the absence of acetyl methyl carbinol resulting from the fermentation (Islam *et al.,* 2016).

The confectionery tests carried out were five types of sugars used, namely glokusa, sucrose, lactose, maltose, mannitol. In sample no. 1 shows that the *Lactobacillus* sp. which grows unable to ferment glucose, sucrose, and lactose which is indicated by the absence of discoloration that is still red, but this bacterium is able to ferment maltose and mannitol with a change in color to yellow. Then in sample no. 2 shows the ability of *Lactobacillus* sp. ferment glucose, sucrose, lactose, and maltose with a marked change in color to yellow, but this bacterium is unable to ferment mannitol, this also occurs in sample no. 3. Then the results in sample no. 4 shows the ability to ferment glucose, sucrose, and maltose, but negative for lactose and mannitol, the difference in fermenting sugars is one of the differentiators of each species of the genus *Lactobacillus* (Tambunan, 2016).

Conclusion

Based on the research that has been done, it can be concluded that there is *Lactobacillus* sp. in the pandan mongoose faeces as evidenced by biochemical tests produce morphology and characteristics that are appropriate and based on the ability of sugar fermentation indicates the presence of species differences.

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