Isolation and characterization of yeast isolated from civet (Paradoxorus hermaphroditus)

Rasdiansyah, M Muzaifa*

Department of Agricultural Product Technology, Faculty of Agriculture Syiah Kuala University - Banda Aceh, Indonesia

*Email: murnamuzaifa@unsyiah.ac.id

Abstract. Civet coffee is one of the most popular coffee in the world. This coffee is fermented naturally in the digestive tract of civet (Paradoxorus hermaphroditus). The coffee fermentation is characterized by the presence of different microorganisms belonging to the groups of yeast, fungi and bacteria. The aim of this work were to isolation and characterization of yeast isolated from civet. The isolation was carried out using Sabouraud Dextrose Agar (SDA). A total of three different yeasts (YC1, YC2 and YC3) were isolated and subjected to morphology and biochemical test. The result showed that all isolates had various characteristics on carbohydrate fermentation, temperature tolerance and proteolityc activity. Two isolates (YC1 and YC2) showed good proteolityc activity. Further investigation is needed to identify and select of yeast performance on coffee fermentation.

1. Introduction
Civet coffee or kopi luwak coffee is known as one of the most popular coffee in the world. This coffee is produced from coffee cherries which have been eaten by civet (Paradoxorus hermaphroditus) and pass through its digestive tract. Indigestible beans are excreted with the civet feces, collected, washed, cleaned, dried, ground and roasted with the same process as regular coffee [1, 2]. Civet coffee is known has better flavor than regular coffee. The unique flavor of civet coffee comes from the digestion process in digestive tract of civet includes mechanical, biochemical and fermentation process [1, 3-5].

Civet coffee is the rarest and the most expensive coffee in the world which is produced around 500-700 kg per year. The demand for this coffee is increase rapidly with the increasing of civet coffee popularity. One of the reasons that civet coffee has high price is because of its limited supply. Civet coffee production is highly dependent on civet biology’s system. On the other hand, existence of civet is diminishing [1, 6, 7].

In vitro fermentation may be an option on civet coffee production. Natural fermentation processes in civet digestive tract changes the chemical composition of coffee beans and improve the taste quality of coffee [3]. In the fermentation process a chemical event occurs which is very useful in the formation of flavor of coffee bean flavor, namely the formation of flavor precursor compounds such as amino acids and reducing sugars. This allegation causes the aroma of civet coffee to be very special [1,3,5]. Fermentation is associated with activity of various microorganisms. Several studies have been conducted to determine the types of microorganism involved in civet coffee fermentation. Microorganisms have been isolated from civet i.e lactic acid bacteria and non-lactic acid bacteria [8-10].
The use of bacteria as starter culture on simulation of civet coffee fermentation was reported [9,12]. There is no information about yeast from civet digestive tract. The existence of yeast in the gastrointestinal tract is still not fully understood. Yeast is unicellular fungi group that reproduces sexually or asexually. As a single cell, yeast is more effective in breaking down chemical components because it has a greater surface area to volume ratio. Yeast has a great potential in giving changes to the taste, aroma and texture of food. It has a lot to play in the fermentation process such as in the manufacture of beer, bread and other fermented foods [13]. The ability of yeast to break down substrates efficiently is a superior characteristic of yeast. Therefore it is necessary to isolate and characterize the yeast obtained from the civet.

2. Methods

2.1. Sample collection

This research was explorative study on isolation and characterization of yeast from civet feces. Sample of civet feces was obtained from a farm civet in Aceh Tengah, Aceh Indonesia. The feces was collected in a sterilized sampling flask, kept in ice box and transported to laboratory.

2.2. Isolation of yeast from civet feces

Yeast was isolated from civet feces by taking one loop of the feces sample into pepton and shakes for 10 minutes on the shaker. About 1 ml of the sample was added to 9 ml of sterile distilled water and this suspension was serially diluted. About 0.1 ml from each dilution was spread plated onto sterile Sabouraud Dextrose Agar (SDA) plates with 1% chloramphenicol (0.05 g/l) and incubated at 30 °C for 48 hours [14]. Different and single colony types were picked up and purified by repeated streaking. Pure cultures were submitted to morphological and biochemical test.

2.3. Morphological and biochemical test

Characterization of yeast isolates was made by following the methods of Barnett et al [15]. Morphological characteristics of the yeasts colony were examined after growth on SDA media. The colony morphology observed was form, color, elevation, margin/edge and size. Biochemical test was performed for temperature tolerance, carbohydrate utilization and proteolitic activity. Temperature tolerance test was conducted by cultured yeast isolates on SDA and incubated at 15, 35 and 45°C for 72 h, growth was observed and analysed. The carbohydrate utilization test was performed using broth containing different carbohydrates. The carbohydrates used were glucose, lactose, manitol, maltose and sucrose. The media were inoculated with yeast strains and incubated for 48 h. The change of colour from purple to yellow indicated that the carbohydrate was using in the fermentation. Proteolitic activity was performed using skim milk agar. Selected yeast strains were spot inoculated on 1% skim milk agar plates and incubated at 30°C for 48 h. The formation of halo (clear) zone indicated the proteolitic activities of bacteria resulting from milk protein hydrolysis [16].

2.4. Data analysis

All data obtained from the microbial analysis represented in table form. Data were analyzed descriptively.

3. Result and Discussion

Based on morphological characteristics, three different colonies were isolated (Table 1). The isolates were vary on form, color, margin and size. Colonies formed by yeast isolates were circular and irregular, white to whitish coloured. Colony size range from 2.86 to 4.93 mm, raised in elevation and their margin were entire to undulate. Individual cells were ovoid to spherical (Figure 1). Photomicropgraph of the isolates were shown in Figure 2.
**Table 1.** Colony characteristics of yeast isolates from civet

| Isolates | Form      | Color            | Margin/edge | Elevation | Size (mm) |
|----------|-----------|------------------|-------------|-----------|-----------|
| YC 1     | Circular  | White            | Entire      | Raised    | 2.86      |
| YC 2     | Irregular | Whitish Cream    | Undulate    | Raised    | 4.13      |
| YC 3     | Circular  | Whitish Cream    | Undulate    | Raised    | 4.53      |

**Figure 1.** Cell characteristic of yeast isolates from civet

**Figure 2.** Photomicrograph of yeast isolates from civet

These three yeasts further used for biochemical characterization. Temperature tolerance, carbohydrates fermentation and proteolitic activity of the isolates were shown from Table 2 to Table 4.

**Table 2.** Temperature tolerance of yeast isolates from civet

| Isolates | Temperature |
|----------|-------------|
|          | 15°C | 35°C | 45°C |
| YC 1     | +    | ++   | -    |
| YC 2     | +    | ++   | -    |
| YC 3     | +    | ++   | -    |

Temperature is one of the most important physical parameters which has an influence on yeast growth and fermentation performance. Table 2 showed that all isolates were able to grow at
temperatures of 10°C and 35°C but could not grow at 45°C. However, the current study indicated that the isolates grew well at 35°C. Although many types of yeast are mesophilic organisms, the preferred temperature for yeasts is between 25 to 35°C [17]. The growth of yeasts that were cultivated at temperatures of 10°C and 15°C was very slow [18].

| Table 3. Sugar fermentation of yeast isolates from civet |
|----------------------------------|
| Isolates | Sugar Fermentation |
|         | Glucose | Lactose | Mannitol | Maltose | Sucrose |
| YC 1    | +       | +       | -        | -       | -       |
| YC 2    | -       | +       | +        | +       | -       |
| YC 3    | -       | +       | +        | -       | -       |

Table 3 showed that yeast isolates have different capacity on sugar fermentation. Sugar fermentation test is used to analyze the ability of isolates to break down or ferment certain sugars in this case glucose, lactose, mannitol, maltose and sucrose. Isolate YC 1 could ferment glucose, lactose and mannitol, isolate YC2 could ferment lactose, mannitol and maltose. Isolate YC3 only could ferment lactose and mannitol. All isolates could not ferment sucrose. During fermentation, organic substrate (sugar) acts as the final electron acceptor with the end result being acids and gases. The main flavor compounds produced by yeast during fermentation were alcohols, esters, organic acids, aldehydes and sulfur compounds [19].

| Table 4. Proteolityc activity of yeast isolates from civet |
|----------------------------------|
| Isolates | Proteolityc activity |
| YC 1    | +                   |
| YC 2    | +                   |
| YC 3    | -                   |

Proteolityc activity was showed by a precipitate (halo zone) around colony after incubation [16]. Table 4 showed that two isolates YC1 and YC2 were able to produce protease. This indicated that the yeasts could break down protein. Proteolityc activity was not detected for YC3 isolate. Although it has been reported that high proteolityc activity is relatively rare in yeasts, the intracellular proteases produced by yeasts have been studied due to their metabolic importance [20-21]. Proteolysis during fermentation led to increase in amino acids concentrations [22]. These proteolityc yeast isolates should be further investigated for identification and the development of a starter culture on coffee fermentation.

4. Conclusions
Three different yeasts were isolated from civet feces. The morphology and biochemical of the isolates were characterized well. The isolates had various characteristics on carbohydrate fermentation, temperature tolerance and proteolityc activity. All isolates were able to grow at temperatures of 10 °C and 35°C but could not grow at 45 °C. Two isolates YC1 and YC2 showed good proteolityc activity. Further investigation is needed to identify and to confirm of yeast performance on coffee fermentation.

Acknowledgements
We would like to thanks Syiah Kuala University for giving us the opportunity to conduct this research under the scheme of “Penelitian Lektor” in the year of 2019.
References
[1] IB Smith. 2014. Kopi luwak coffee-world’s most expensive coffee beans from civet poop or an urban myth? IBS Publishing, USA.
[2] M Muzaifa, A Patria, Febriani, A Abubakar A, D Hasni, F Rahmi , I Sulaiman. 2016. Kopi luwak produksi mutu dan permasalahannya. Syiah Kuala University Press.
[3] M Marcone. 2004. Composition and properties of Indonesian palm civet coffee and Ethiopian civet coffee. Food Res. Int. 37: 901-912.
[4] M Cuang-Hoang. 2012. Method for processing coffee and coffee processed by this method, Patent WO 2012009730A1, Jan 19.
[5] Yusianto, S. Mawardi, C. Ismayadi dan Sulistyowati. 2010. Kopi Luwak: Karakteristik fisik dan kimia. Simposium Kopi 4-5 Oktober, Bali.
[6] Panggabean, E. 2011. Buku Pintar Kopi. AgroMedia Pustaka, Jakarta.
[7] Sucipto. 2010. Penguatan Citra Kopi Luwak di Indonesia. Universitas Brawijaya.
[8] S Suhandono, H Setiadi, T Kristianti , AB Kusuma, W Wedanringtyas, DT Djajadi, INP Aryantha.2016. Diversity of culturable bacterial in various parts of luwak’s (Paradoxurus hermaproditus Javanica) gastrointestinal tract," Microbiol. Indonesia 10 (2):65-70.
[9] M Hadipernata dan S. Nugraha, 2012. Identifikasi sifat fisik, kimia dan mikrobiologi biji kopi luwak sebagai dasar acuan teknologi proses kopi luwak artificial. Prosiding InSinNas 29-30 November, Jakarta.
[10] M Muzaifa, A Patria, A. Abubakar. 2016. Isolation and Screening of Proteolytic Lactic Acid Bacteria from Civet. The 6th Annual International Conference Syiah Kuala University (AIC Unsyiah) in conjunction with The 12th International Conference on Mathematics, Statistics and Its Application (ICMSA) Banda Aceh, October 2016
[11] M Fauzi. 2008. Isolasi dan karakterisasi bakteri asam laktat biji kopi luwak (civet coffee). Laporan Penelitian, Universitas Jember.
[12] S Guntoro. 2010. Proses memproduksi kopi luwak probiotik (The process of producing probiotic civet coffee). Patent. Balai Pengkajian Teknologi Pertanian (BPTP) Bali, Denpasar Indonesia.
[13] AK Rai and J Kumaraswami. 2017. Role of Yeasts in Food Fermentations. In Yeast Diversity in Human Welfare.
[14] RD Cagno, E Pontonio, S Buchin, MD Angelis, A Lattanzi, F Valerio, M Gobbetti, M Calasso. 2014. Diversity of the Lactic Acid Bacterium and Yeast Microbiota in the Switch from Firm- to Liquid-Sourdough Fermentation. Applied and Environmental Microbiology 80(10): 3161–3172
[15] JA Barnett, RW Payne, D Yarrow. 2000. Yeasts: Characteristics and identification. 3rd Edition, Cambridge University Press, UK.
[16] VM Cardoso, BM Borelli, CA Lara, MA Soares, C Pataro, EC Bodevan, CA Rosa. 2015. The influence of seasons and ripening time on yeast communities of a traditional Brazilian cheese. Food Research International, 69: 331–340
[17] K Watson. 1987. Temperature relations. In The Yeasts. Vol. 2 (eds A.H. Rose and J.S. Harrison), 41-72. Academic Press, London.
[18] DHN Ho and C. Powell. 2014. The Effect Temperature on the Growth Characteristics of Ethanol Producing Yeast Strains. International Journal of Renewable Energy and Environmental Engineering 2(1): 1-6.
[19] MC Dzialo, R Park, J Steensels, B Lievens and KJ Verstrepen. 2017. Physiology, ecology and industrial applications of aroma formation in yeast. FEMS Microbiol. Rev. 41:S95-S128
[20] M Poza, T Miguel, C Siero, TG Villa. 2001. Characterization of a broad pH range protease of Candida caseinolytica. Journal of Applied Microbiology, 91(5): 916-921
[21] G Koelsch, J Tang, JA Loy, M Monod, K Jackson, X Foundling. 2000. Enzymic characteristics of secreted aspartic proteases of Candida albicans. Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology, 480 (1-2): 117-131.
[22] LW Lee, MW Cheong, P Curran, B Yu, SQ Liu. 2016. Modulation of coffee aroma via the fermentation of green coffee beans with Rhizopus oligosporus: I. Green coffee. Foodchem 211:916-24.