ACID-PRODUCING BACTERIA AND YEASTS ISOLATED FROM ARABICA COFFEE PLANTATIONS IN CENTRAL JAVA AND EAST JAVA, INDONESIA

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Abstract

The converting process of coffee cherries to green coffee beans before roasting was conducted through fermentation using lactic acid bacteria (LAB) and yeasts to degrade coffee's mucilage. This present study aims to isolate acid-producing bacteria and yeasts from three types of samples obtained from arabica coffee plantations in Central Java and East Java, Indonesia. One sample from feces of luwak or civet (*Paradoxurus hermaphroditus*), two samples from exocarp of arabica's coffee fruits, and one sample from the soil in the rhizosphere of a coffee tree. Enrichment method by using MRS broth supplemented with 0.5% calcium carbonate was used for obtaining acid producing bacteria and yeasts. Phylogenetic tree based on 16S rRNA gene for bacteria and 26S rRNA gene for yeast were constructed and analyzed. The screening of their ability to produce acid was conducted by using MRS agar supplemented with 0.5% calcium carbonate. A total of 31 bacteria and 15 yeasts were obtained from this study. From 31 bacteria, 15 strains were identified as *Pediococcus pentosaceus,* 6 strains as *Lactobacillus* sp., 4 strains as *Leuconostoc pseudomesenteroides,* 5 strains as *Acetobacter tropicalis*; and 1 strain as *Klebsiella quasipneumoniae*. While from 15 yeasts, 8 strains were identified as *Candida tropicalis*, 2 strains as *Pichia kudriavzevii*, and 5 strains as *Cyberlindnera fabianii*. A total of 25 strains of lactic acid bacteria were able to release lactic acid on MRS agar supplemented with 0.5% $CaCO_3$, but 5 strains of *A. tropicalis* and 1 strain of *K. quasipneumoniae* produced acid weakly. Four strains of *C. tropicalis* showed the ability to form a thin clear zone in contrast to the other of four yeast strains. This study revealed that samples from the coffee plantation areas are potential sources to obtain acid-producing bacteria which can be utilized in fermentation process of coffee cherries.

Keywords: arabica coffee, acid-producing bacteria, acid-producing yeast

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Introduction

Coffee is a crucial agriculture commodity in tropical countries, particularly in Indonesia, and the most valuable exchanged commodity in international market, which is regularly consumed globally as a non-alcoholic drink and caffeine-containing beverage (Haile & Kang, 2019; Vaughan *et al*., 2015). Fitriani *et al*. (2021) reported that Indonesia is the 4th largest coffee producer and exporter globally, just after Brazil, Vietnam, and Colombia. *Coffea arabica* (arabica coffee) and *Coffea canephora* var. *robusta* (robusta coffee) are two valuable coffee types sold in the global market and consumed throughout the world. These coffee types dominate coffee production worldwide, as much as 60% for arabica coffee and 40% for robusta coffee (Van der Vossen et al., 2015). In Indonesia, the largest robusta producing regions are centered in southern Sumatera, covering Lampung, South Sumatera, and Bengkulu provinces and small amount in Java. Arabica production is concentrated in northern Sumatera, primarily in Aceh and North Sumatera provinces and several highland areas

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in Java (USDA, 2021). Moreover, the Ministry of Trade Republic of Indonesia (2014) reported that Indonesia is known as the world's best producer of arabica coffee, labeled as "Java coffee", a high-quality coffee in international market.

Post-harvest processing of coffee cherries for obtaining green coffee beans before roasting is carried out through fermentation process (De Bruyn *et al*., 2017). This process uses sugar or other carbon sources in the absence or presence of oxygen to decrease the coffee beans' water content and remove coffee's mucilage that contains polysaccharides (pectin), cellulose, and starch (Haile & Kang, 2019). Previous studies have reported that fungi, yeasts, and bacteria species, mainly lactic acid bacteria, play a role in degrading mucilage by releasing various enzymes, ethanol, lactic, butyric, and acetic acid during coffee fermentation (de Melo Pereira *et al*., 2014; Haile & Kang, 2019; Silva *et al*., 2000; Silva *et al*., 2013).

Lactic acid bacteria (LAB) are heterogeneous group of bacteria that play a significant role in a variety of fermentation processes with the primary function of producing lactic acid (Bintsis, 2018). These bacteria contribute to the development of aroma, flavor, acidity (by producing organic acids), texture of the coffee products, and reduce fermentation time during the fermentation process (Haile & Kang, 2019; Martinez *et al*., 2019; Pereira *et al*., 2016; Wang *et al*., 2018).

Yeasts have been reported to improve coffee quality by producing unique aromatic compounds (de Melo Pereira *et al*., 2014), such as caramel, herbs, and fruits (Evangelista *et al*., 2014), releasing pectin degrading enzymes (Masoud & Jespersen, 2006), and increasing antioxidant activity and flavor of coffee during coffee fermentation (Kwak *et al*., 2018).

Several studies reported that lactic acid bacteria and yeasts are widespread in dairy food, fermented food, meat, vegetable, gastrointestinal and urogenital tracts of humans and animals, soil, and water (Ranadheera *et al*., 2017; Ruiz Rodríguez *et al*., 2019; Zommiti *et al*., 2018).

The utilization of indigenous lactic acid bacteria and yeasts can improve coffee beans' quality during its fermentation process to increase the quality, amount of production, and selling price of arabica coffee beans in the global market. However, studies concerning lactic acid bacteria and yeasts' sources from luwak or civet (*Paradoxurus hermaphrodithus*) feces, coffee waste (exocarp of coffee beans), and the soil of arabica coffee plantations are still limited, particularly in Indonesia which is known as luwak coffee producer, the most expensive coffee in the world. A study showed that lactic acid bacteria isolated from luwak feces improve the quality of coffee's fermentation (Usman *et al*., 2015). Hence, the lactic acid bacteria and yeasts isolated from luwak feces are expected to be able to increase the amount of coffee beans production and enhance the *in vitro* fermentation process (outside the luwak body) with a coffee quality equivalent to the fermentation process in its digestive system. Therefore, this present study aims to obtain not only lactic acid bacteria, but also several indigenous acid-producing bacteria and yeasts from coffee plantation to improve the quality and quantity and increase the amount of coffee beans production during arabica coffee cherries' fermentation process.

Materials and Methods

Materials and Sampling Locations

Four samples were collected from 2 locations of arabica coffee plantation area in Java Island, Indonesia. Three samples were collected from an arabica coffee plantation in Wonosobo Regency, Central Java, Indonesia which consisted of luwak fresh feces containing arabica coffee cherries, exocarp of coffee cherries, and soil of the coffee plantation. Then, one sample of exocarp of coffee cherries was collected from the Dampit sub-district, Malang Regency, East Java, Indonesia.

Isolation and Preservation of Acid-Producing Bacteria and Yeasts

Half gram of each sample was enriched in de Man, Rogosa, and Sharpe (MRS) broth (Oxoid) media and incubated for 72 h at 30ºC with shaking at 150 rpm (Xiao *et al*., 2015). A portion of 200 µL of serial dilution of these medium was put inside a sterile petri dish then was poured by MRS agar medium supplemented with 0.5% $CaCO_3$ (Meidong *et al*., 2017). The pH of the medium was adjusted to 5.5-5.9. All plates were incubated at 30ºC for 6 days in the incubator. Then, the colonies with divergent morphology inside the agar and on the agar surface were selected randomly and

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purified by replicating on MRS agar plates (Chen *et al*., 2010). Pure colonies were preserved in 1.2 mL solution containing 10% glycerol for bacteria and in 1.3 mL solution containing 10% glycerol and 5% trehalose for yeasts. All isolates were stored at 4ºC overnight and then preserved at -80ºC.

Screening of Acid-Producing Bacteria and Yeasts

The acid-producing bacteria and yeasts were qualitatively screened by examining these isolates for a clear zone formation by replating and growing on MRS agar supplemented with 0.5% $CaCO_3$ (Wang *et al*. 2014;Chen *et al*., 2006).

Molecular Identification Based on 16S rRNA and 26S rRNA Gene Sequencing

A colony of each bacterium and yeast grown overnight on MRS agar was picked by using a sterile wooden toothpick to a microtube containing 15 µL of sterile distilled water, mixed homogenously and then boiled at 98ºC for 10 minutes on a block heater (Stuart) (Fukui & Sawabe, 2007). Colony PCR was performed using Arktik Thermal Cycler (Thermo Scientific) to amplify 16S rRNA gene sequence for bacteria (Iswanto *et al*., 2019) and 26S rRNA gene sequence for yeasts (Kanti & Sumerta, 2016).

Construction of Phylogenetic Tree

DNA sequencing of 16S rRNA bacterial gene and 26S rRNA yeast gene were performed by Macrogen Laboratories (South Korea). The sequencing results were analyzed using BioEdit (7.2.5) software (Hall, 1999). The identification of bacterial and yeast gene was performed by comparing the sequence obtained from 16S rRNA gene sequencing to in the public database EzTaxon (https://www.ezbiocloud.net/) (Kim *et al*., 2012) for bacteria and NCBI Genebank using BLAST (https://blast.ncbi.nlm.nih.gov/) for yeasts (Altschup *et al*., 1990; Ladunga, 2009). The phylogenetic tree of bacteria and yeasts was constructed using the neighbor-joining method with p-distance (NJp) with 1000 replicates using MEGA7 software (Saitou & Nei, 1987; Kumar *et al*., 2016).

Results

Isolation of Acid-Producing Bacteria and Yeasts

Forty-six strains were collected from 4 different samples from two coffee plantation areas in Java Island using enrichment culture techniques. These isolates consisted of 31 stains of bacteria and 15 strains of yeasts (Table 1), that grew well on MRS agar medium. All the bacteria were deposited in Indonesia Culture Collection (InaCC) with accession number InaCCB1375–B1399 and InaCC B1427–1432.

Table 1. The number of isolates from four samples of arabica coffee plantation

Sampling location	Sampling source	∑ of samples	∑ of bacteria	∑ of yeasts
Wonosobo, Central Java	Luwak feces in the cage	1	1	3
	Exocarp of Arabica coffee cherries I	1	18	3
	The soil in the rhizosphere of the coffee plantation	1	4	8
Malang, East Java	Exocarp of Arabica coffee cherries II	1	8	1
Total		**4**	**31**	**15**

In this study, isolates that grew under and on the agar surface of MRS medium with off-white pin head colonies and capable of forming a clear zone around the colony, were picked and purified. Morphological observations of these isolates showed some differences in the color and the size amongst the colonies. Almost white and brown colonies dominated these isolates, then variation in colony size between bacteria and yeast colony showed a difference (Figure 1).

Qualitative Screening of Lactic Acid-Producing Bacteria and Yeasts

Twenty-six bacteria and 4 yeasts showed a clear zone formation ability around the colonies on the medium, which indicated the ability of these isolates to release acid and dissolve $CaCO_3$ (Table 2). However, five bacteria and 11 yeasts were weak in releasing acid. Five strains of bacteria (InaCC B1429-B1432 and InaCC B1427) obtained from exocarp of the coffee cherries were weakly able to form a clear zone

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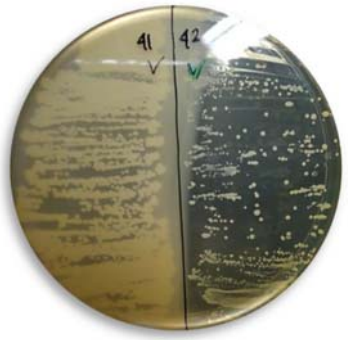
around the colony, similar to the 11 yeasts isolated from all samples.



A B

Figure 1. Variation of yeast and lactic acid bacteria morphology (size and color of the colonies) in MRS agar medium with 0.5% $CaCO_3$. A: LY 41 (yeast) and B: InaCC B1387 (lactic acid bacteria).

Molecular Identification of Acid Producing Bacteria

The bacteria strains were identified based on 16S rRNA gene sequence and analyzed by comparing the sequence to public database. The molecular identification was supported by the phylogenetic tree analysis based on a neighbor-joining method. The phylogenetic tree of 31 bacteria was divided into two phylogenetic trees based on phyla (Figure 2 and Figure 3). A total of 25 isolates belongs to the Phylum *Firmicutes* (80.6%), which consists of genera *Pediococcus* (48.3%, 15 strains), *Lactobacillus* (19.3%, 6 strains), and *Leuconostoc* (13%, 4 strains). Moreover, five strains belong to the Phylum *Proteobacteria* (19.4%) which consists of genera *Acetobacter* (16.1%, 5 strains) and *Klebsiella* (3.3%, 1 strain).

Table 2. Qualitative screening of acid-producing bacteria and yeasts isolated from Luwak feces, exocarp of Arabica's coffee cherries, and soil

Sample source	InaCC Accession Number	The number of isolates	Screening for acid-producing (Clear zone formation)
Luwak feces	*Lactobacillus*: InaCC B1394.	1	++
	Cyberlindnera: LY 1. *Pichia*: LY 2, LY 5.	3	w
Exocarp of coffee cherries	*Pediococcus*: InaCC B1379, InaCC B1380, InaCC B1381, InaCC B1382, InaCC B1383, InaCC B1384, InaCC B1385, InaCC B1386, InaCC B1387, InaCC B1388, InaCC B1389, InaCC B1390, InaCC B1391, InaCC B1392, InaCC B1393. *Acetobacter*: InaCC B1428. *Lactobacillus*: InaCC B1399. *Leuconostoc*: InaCC B1375, InaCC B1376, InaCC B1377, InaCC B1378.	21	++
	Acetobacter: InaCC B1427, InaCC B1429, InaCC B1430, InaCC B1431. *Klebsiella*: InaCC B1432.	5	w
	Cyberlindnera: LY 20, LY 41, LY 43, LY 45.	4	w
Soil	*Lactobacillus*: InaCC B1395, InaCC B1396, InaCC B 1397, InaCC B1398.	4	++
	Candida: LY 7, LY 11, LY 13, LY 14.	4	+
	Candida: LY 6, LY 12, LY 15, LY 17.	4	w
	Total	**46**	

Note: ++, strong on producing acid; +, positif on producing acid; w, weak on producing acid.

The 15 strains of LAB were closely related to *Pediococcus pentosaceus* with bootstraps 95% (Figure 2) and were only found in the exocarp of coffee cherries samples and strong on producing acid. Six isolates released lactic acid were closely related to species *Lactobacillus* sp., among them strain InaCC B1394 were obtained from luwak feces, strains InaCC B1395-B1398 from soil in Wonosobo District, and strain InaCC B1399 from exocarp of coffee from Dampit, Malang (Table 2, Figure 2). Four strains (InaCC B1375-B1378) belong to the genus *Leuconostoc* were closely related to *Leuconostoc pseudomesenteroides* with

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bootstraps 84% (Figure 2), only existed in exocarp of coffee sample from Magelang, and strong on producing acid.

A total of 5 isolates belonging to the Phylum *Proteobacteria* (Figure 3) were distributed among two genera (*Acetobacter* and *Klebsiella*). Strains InaCC B1427-B1431 were identified as *Acetobacter tropicalis*, and strain InaCC B1432 was identified as *Klebsiella quasipneumoniae* with bootstraps value 99% (Figure 3). *Acetobacter tropicalis* was only obtained from the exocarp of coffee cherries samples. Only one isolate of *A. tropicalis* showed a clear zone formation on agar media containing $CaCO_3$ (InaCC B1428). Genus *Klebsiella*, the second genera from Phylum *Proteobacteria* found in this present study, was only obtained from the exocarp of coffee cherries from Dampit, Malang.

Molecular Identification of Yeasts

Molecular identification of yeasts was based on the sequencing results of the 26S rRNA gene. Hereafter, the phylogenetic tree (Figure 4) shows that 15 yeast isolates belonging to the phylum Ascomycota (100%), which includes the following genera: *Candida* (53.3%, 8 strains), *Cyberlindnera* (33.3%, 5 strains), and *Pichia* (13.3%, 2 strains).

Two isolates belonging to the genus *Pichia* were obtained from luwak feces from Wonosobo and were only found in feces samples. The phylogenetic tree (Figure 4) showed that strains LY 2 and LY 5 were closely related to *Pichia kudriavzevii* with bootstraps 100% by the neighbour-joining method. Five (LY 1, LY 20, LY 41, LY 43, LY 45) isolates belonging to genus *Cyberlindnera*. These isolates were closely related to *Cyberlindnera fabianii*. Four strains were isolated from the exocarp of coffee cherries from Wonosobo and Dampit without the ability to produce acid and one strain was isolated from feces of luwak without the ability or weak to produce acid.

The last yeast genera isolated from this study was eight isolates from the soil sample belonging to the genus *Candida*, a predominant yeast obtained in the present study and showed a specific species to soil sample. Four yeasts (LY 7, LY 11, LY 13, LY 14) isolated from soil samples which closely related to *Candida tropicalis* (Figure 3), showed a thin clear zone on MRS agar with 0.5% $CaCO_3$. However, other four isolates (LY 6, LY 12, LY 15, LY 17) identified as *C. tropicalis* were unable to form a clear zone. The presence of a clear zone around *C. tropicalis* colonies indicated that this yeast species dissolved calcium carbonate.

Discussion

The isolation method in this present study used enrichment techniques in MRS broth to obtaining 46 strains that consisted of 31 strains of bacteria and 15 strains of yeasts from 4 samples. MRS media has generally been used to isolate lactic acid bacteria (Carr *et al*., 2002). However, this media was incapable of preventing yeasts' growth (Brocklehurst & Lund, 1984; Rose, 1985). Thus, various genera of bacteria and yeasts were obtained during isolation by using this media. During isolation, isolates were picked up randomly based on the different colony size and clear zone formation for obtaining only lactic acid bacteria instead of yeasts. However, Rose (1985) reported that it is impossible to visually differentiate yeasts or *Lactobacillus* colonies on MRS agar and prevent yeast growth on MRS agar even though antibacterial is added to agar media.

Calcium carbonate ($CaCO_3$) in the MRS agar was added to qualitatively screen for acid production. The present study showed that 26 bacteria and 4 yeasts formed a clear zone and indicated their ability to dissolve $CaCO_3$. Lactic acid bacteria dissolve calcium carbonate by releasing lactic acid, decreasing the pH of the medium, increasing $CaCO_3$ solubility, and subsequently forming a clear zone in the agar medium (Lawalata *et al*., 2015). However, 5 acetic acid bacteria, 1 *Klebsiella*, and 11 yeasts also dissolve calcium carbonate, but not as strong as lactic acid bacteria. Calcium carbonate is not only used to isolation lactic acid bacteria and screen their ability to produce lactic acid. Lisdiyanti *et al*. (2000, 2001) used $CaCO_3$ to isolate acetic acid bacteria in pH 3.5.

Pediococcus, *Lactobacillus*, and *Leuconostoc* are Gram-positive bacteria and produce lactic acid that belong to the order *Lactobacillales* (Sun *et al*., 2014). Therefore, it is not surprising that 25 strains belong to these genera can form a clear zone by producing and releasing lactic acid and dissolving $CaCO_3$, among them fifteen strains were closely related to *P. pentosaceus,* six strains were closely related to *Lactobacillus* sp., and four strains were closely related to *L. pseudomesenteroides*.

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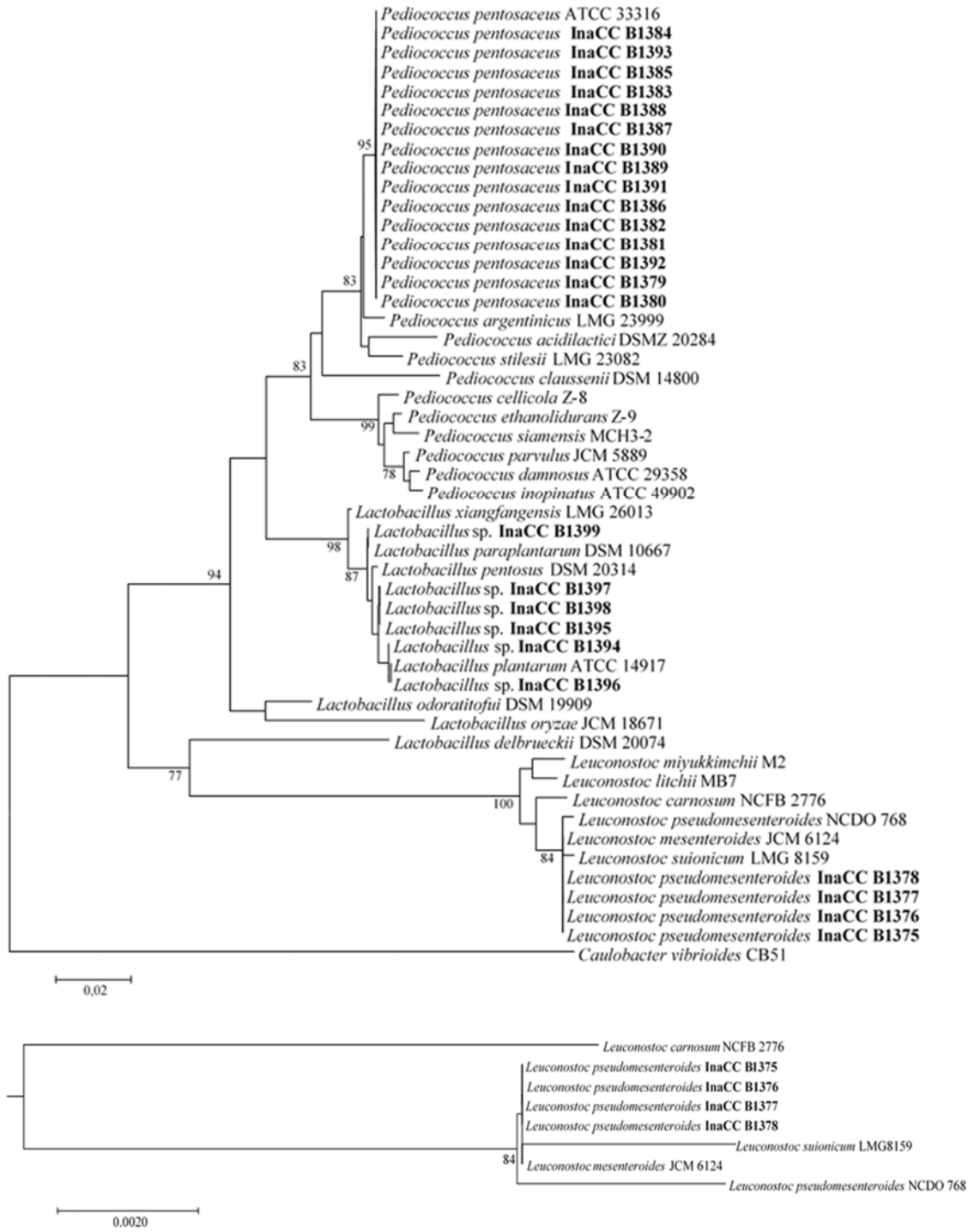

Figure 2. The phylogenetic tree of Phylum *Firmicutes* based on 16S rRNA gene sequencing. A tree was constructed by using MEGA 7 software with Neighbor-Joining method and evolutionary distances using p-distance method (NJ-p) with 1000 replicates. The bootstraps above 70% was showed. Species *Caulobacter vibrioides* CB51 was used as outgroup.

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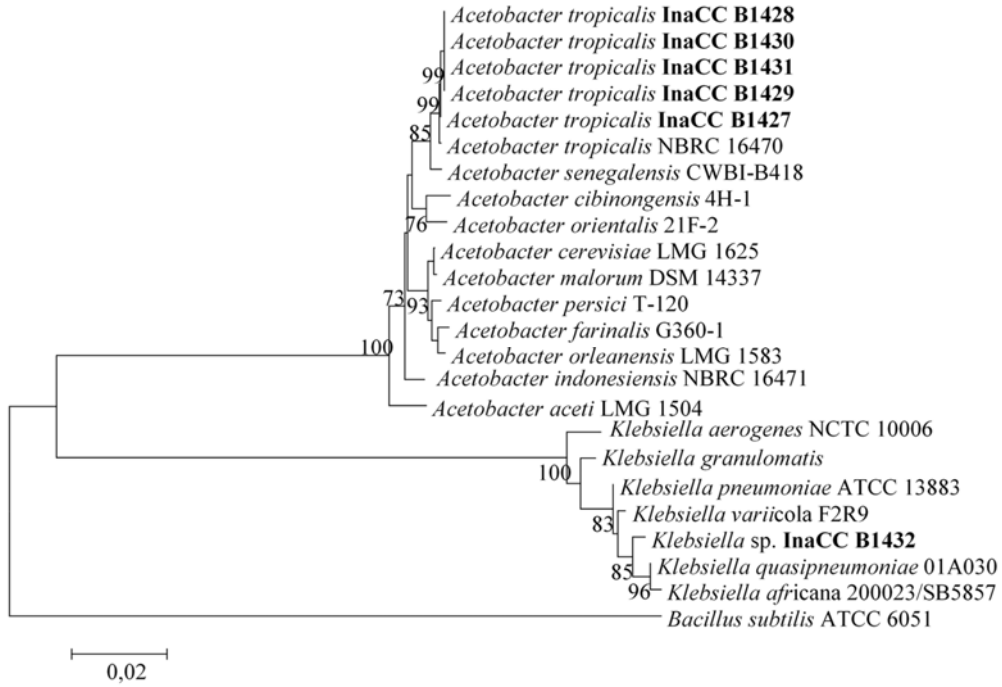

Figure 3. The phylogenetic tree of Phylum *Proteobacteria* based on 16S rRNA gene sequencing. A tree was consisted of two genera (*Acetobacter* and *Klebsiella*) and constructed by using MEGA 7 software with Neighbor-Joining method and evolutionary distances using p-distance method (NJ-p) with 1000 replications. The bootstraps above 70% was showed. *Bacillus subtilis* ATCC 6051 was used as outgroup.

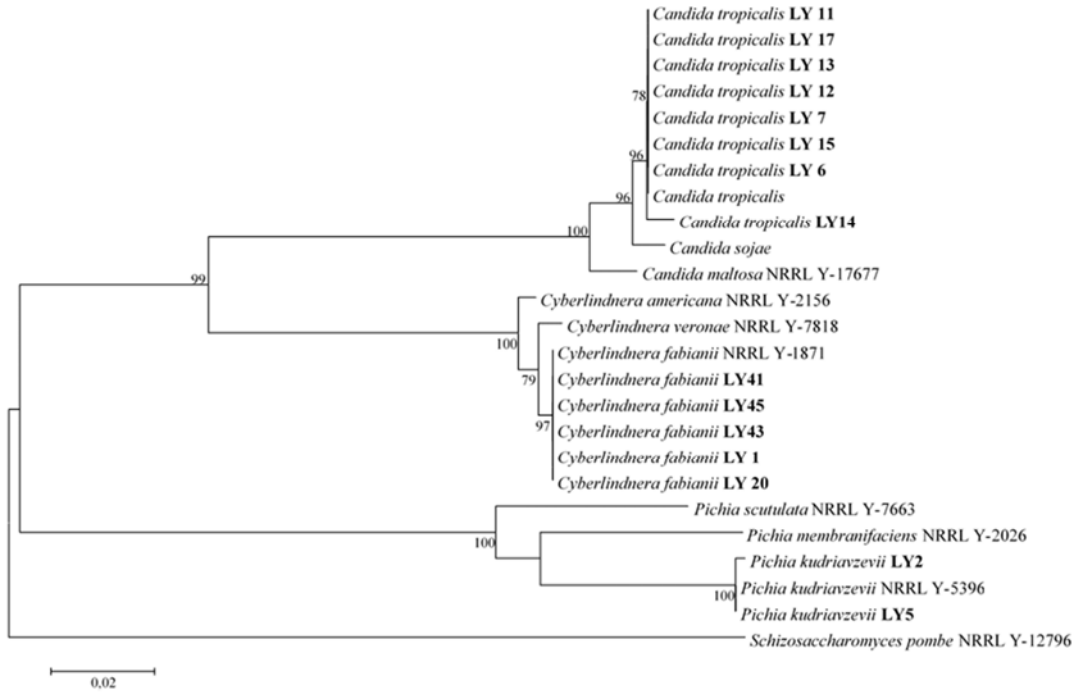

Figure 4. The phylogenetic tree of three genera of yeasts based on 26S rRNA sequencing. The tree was constructed by MEGA 7 software using Neighbor-Joining method and evolutionary distances using p-distance method (NJ-p) with 1000 replicates. The bootstraps above 70% was showed. Species *Schizosaccharomyces pombe* NRRL Y-12796 was used as outgroup.

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Fifteen strains produced acid were closely related to *P. pentosaceus*. All strains were only obtained from exocarp of coffee samples. There is a limited number of studies regarding the existence of *P. pentosaceus* in coffee. This species was first found in Ecuador's coffee bean fermentation (de Melo Pereira *et al*., 2020). *Pediococcus pentosaceus* was a dominant species of LAB obtained in the current study. It may seem that *P. pentosaceus* was found in specific coffee samples, particularly in exocarp of coffee cherries.

This study showed that *Lactobacillus* sp. widely spread in different samples. Several studies have reported that species from the genus *Lactobacillus* existed in luwak feces (Muzaifa *et al*., 2019; Suhandono *et al*., 2016), mature coffee cherries (Silva *et al*., 2000), coffee beans (de Melo Pereira *et al*., 2016), and coffee waste (Montoya *et al*., 2019). However, there is still a limited number of reports on *Lactobacillus* species from the exocarp of coffee cherries in Indonesia.

In this study, four strains closely related to *L. pseudomesenteroides* were isolated from exocarp of coffee sample with ability to produce acid and form clear zone formation. *L. pseudomesenteroides* has been reported abundant in fresh arabica's coffee cherries in Taiwan (Leong *et al*., 2014), coffee pulp from a local coffee plantation in West Java, Indonesia (Oktaviani *et al*., 2020), tropical fruits and vegetables located in Reunion island (Fessard & Remize, 2019), traditional fermented milk of Tibet in China (Airidengcaicike *et al*., 2010), ripe mulberries in Taiwan (Chen *et al*., 2010), cane juice, a pressed sugarcane (Kim *et al*., 2011), and human blood (Tholpady *et al*., 2010). All strains of the genus *Leuconostoc* showed clear zone on MRS agar supplemented with $CaCO_3$. Genus *Leuconostoc* produces lactic acid as a major metabolite of sugar in its fermentation process (Juven *et al*., 1985). *L. pseudomesenteroides* is a member of LAB species found in fresh coffee cherries (Oktaviani *et al*., 2020) and has been reported as dominant species at the early stages of coffee fermentation (De Bruyne *et al*., 2007). This present study showed that this species was only found in exocarp of coffee cherries from Wonosobo, Central Java. Leong *et al*. (2014) reported that *L. pseudomesenteroides* specifically found in fresh coffee cherries grown on-farm located at an altitude in the range of 700-800 m.

The genus *Acetobacter* has been commonly known as the main acetic acid bacteria for aerobic acetic fermentation (Kounatidis *et al*., 2009; Lisdiyanti *et al*., 2003). It oxidizes ethanol to acetic acid in a neutral and acid condition, oxidizes acetate and lactate to CO_2 and H_2O (Jimenez-Salgado *et al*., 1997), and produces clear zones on basal plates containing $CaCO_3$ (Lisdiyanti *et al*., 2003). Interestingly, there is still no report regarding the existence and the ability of *A. tropicalis* in coffee, particularly its ability to release acid. Jimenez-Salgado *et al*. (1997) reported an unsuccessful attempt to isolate *Acetobacter* from coffee cherries, even though it has been reported that *Acetobacter* existed in coffee plant tissues and rhizosphere soil around the coffee root. The maturity of coffee cherry samples may affect the isolation results. The present study showed that one species of the genus *Acetobacter* was successfully isolated from a soil sample, contrary to previous studies reported (Jimenez-Salgado *et al*., 1997).

Avallone *et al*. (2002) reported that *Klebsiella* is redundant and found in coffee fermentation during maturation and processing of coffee cherries of *Coffea arabica* in Brazil (Silva *et al*., 2000), semi-dry coffee fermentation (Evangelista *et al*., 2014), and in the coffee beans from Indonesia (Iswanto *et al*., 2019). These previous studies showed that the genus *Klebsiella* is commonly found in arabica coffee and as we know, the genus *Klebsiella* is able to produce acid weakly.

Eight yeasts obtained from soil sample were closely related to species *Candida tropicalis*. Four yeasts showed ability to release acid and dissolve calcium carbonate. However, four yeasts were unable to produce acid. However, there is still no report on the ability of *C. tropicalis* to produce acid. Several previous studies reported *C. tropicalis* species has been isolated from soil samples (Amprayn *et al*., 2012) and traditional sorghum beer (N'Guessan *et al*., 2016).

Tree strains were closely related to genus *Cyberlindnera* that obtained from exocarp of coffee from two locations. However, this genus was unable to release acid. *C. fabianii* previously known as *Lindnera fabianii*, *Hansenula fabianii*, *Pichia fabianii*, and *Candida fabianii* (Rijswijck *et al*., 2017; Freel *et al*., 2014). Previous studies isolated *C. fabianii* from patients' urine and reported it as infectious yeast that causes a rare human

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infection (Park *et al*., 2019). *Cyberlindnera fabianii* and *P. kudriavzevii* have also been found in fermented *masau* fruits in Zimbabwe, fermented fruit products (Nyanga *et al*., 2007), and water treatment (Freel *et al*., 2014). Nevertheless, *P. kudriavzevii* and *C. fabianii* are considered as promising yeasts for food fermentation applications, such as for producing food aroma (Rijswijck *et al*., 2017).

Two *Pichia* species from luwak feces sample were closely related to *P. kudriavzevii*. Previously, *P. kudriavzevii* was named as *Issatchekia orientalis* (Kurtzman *et al*., 2008) and *Candida krusei* (Douglass *et al*., 2018) and has been reported to be found in food (Chelliah *et al*., 2016), fermented materials (Hong *et al*., 2018) such as fermented masau fruits (Rijswijck *et al*., 2017), dairy products (Rahbar Saadat *et al*., 2020), and human faecal (Madeeha *et al*., 2016). Therefore, this present study is the first report of *P. kudriavzevii* isolated from luwak feces. Both strains obtained from this current study were unable to release lactic acid while *P. kudriavzevii* has been reported as probiotic yeast similar to LAB by Chelliah *et al*. (2016) and Rahbar Saadat *et al*. (2020). Hereafter, *P. kudriavzevii* has been reported both as an opportunistic pathogen in human and beneficial for industry (Douglass *et al*., 2018). Food and Drugs Administration (FDA) issued the status recognition of these species as safe microbes in food applications (Rijswijck *et al*., 2017).

This study revealed that samples from the coffee plantation areas are potential sources to obtain acid-producing bacteria. Bacteria and yeasts obtained in this study showed their ability to produce acid and potential to be utilized in fermentation process of coffee cherries to improve quality of coffee bean. Then, the further study is needed to determine what types of acids are produces by bacteria and yeast isolates and quantitatively analysis of isolates in release acid.

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