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Bacterial and yeast optimization in Arabica coffee fermentation to enhance winey flavor

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Abstract. *Taufik I, Syachnoormalieta FI, Istiadi KA, Rusdiana FF, Astuti DI. 2024. Bacterial and yeast optimization in Arabica coffee fermentation to enhance winey flavor. Biodiversitas 25: 4265-4274.* The natural fermentation process of Arabica coffee often needs more consistency in producing high-quality coffee due to the process's challenging control involving diverse microbial communities, including those of bacteria and yeast. This study has isolated specific bacteria and yeast strains from natural fermentation and utilized them as inoculum to optimize the coffee fermentation process, accentuating the wine's aromatic flavors by adjusting the bacteria-to-yeast ratio and inoculum percentage. Eight bacteria and five yeast were initially isolated from natural fermentation processes and further assayed for their enzymatic activity. The highest enzymatic activity, including pectinolytic, cellulolytic, amylolytic, and proteolytic activities from each bacterial and yeast, were selected for identification using DNA sequencing and phylogenetic tree construction. The identification results showed that the bacteria *Klebsiella* sp. and the yeast were *Pichia kudriavzevii*. Next, the growth curves of the two isolates were analyzed to determine the optimal fermentation time. The results indicated that one day was sufficient for optimal bacterial and yeast growth. Using the isolates bacteria *Klebsiella* sp. and yeast *Pichia kudriavzevii* revealed that a bacteria-to-yeast ratio of 1:10 with a 15% (v/w) inoculum resulted in the highest cupping score with a prominent wine flavor note compared to the control. Employing bacterial and yeast inoculum in Arabica coffee fermentation has the potential to improve coffee quality and consistently improve overall product standards.

Keywords: Enzymatic; *Klebsiella*, *Pichia kudriavzevii*, specialty coffee, wine flavor

INTRODUCTION

Indonesia is the ninth-largest producer of Arabica coffee, with a yield of around 1,541 tons in 2002 (ICO 2023). Arabica coffee is highly consumed worldwide and valued for its flavor, smoothness, acidity, unique taste, and overall balanced attributes (Cordoba et al. 2020; Bastian et al. 2021; Girma and Sualeh 2022). Arabica coffee is favored for its high acidity level compared to Robusta coffee (Pereira et al. 2017). Some coffee drinkers enjoy the wine-like flavor of certain Arabica coffee types due to a specific fermentation process (Zhao et al. 2023). Unfortunately, the inconsistency in this fermentation process often hinders the attainment of the desired wine-like flavors in the coffee.

Inconsistencies in coffee quality can often be attributed to various factors, such as harvesting under-ripe coffee cherries and the processing techniques employed (Pereira et al. 2014). One process in Arabica coffee varieties, which still vary greatly, is natural fermentation, which is greatly influenced by microorganisms (Pei and Schmidt 2018; Madigan et al. 2019). The microorganisms that contribute to the fermentation process exhibit significant diversity and abundance, leading to inconsistency in flavor and often preventing the desired winey flavor (Wu et al. 2024). Wet and dry fermentation methods affect the bacterial and yeast consortia, influencing the coffee's quality and unique taste (Guevara et al. 2024). Additionally, temperature conditions during fermentation also impact the quality and flavor of the coffee (Peñuela-Martínez et al. 2023).

The fermentation stage aims to develop the wine's flavor characteristics. In the drying process, the coffee cherries are dried in the sun without the pulping process to remove the mucus layer on the coffee berries (Bressani et al. 2021; Chang et al. 2024). Enzymes such as pectinase, cellulose, protease, and amylase hydrolyze the substrates in the coffee cherries, with microorganisms expediting these reactions (Milić et al. 2023). Naturally, microorganisms in coffee production utilize various compounds from cherry pulp and mucilage as nutrients during fermentation (Maicas 2020; Ferreira et al. 2023). These microorganisms produce organic acids and other metabolic compounds that significantly impact the final sensory profile of the coffee (Vaz et al. 2023).

Various bacterial, yeast, and fungal microorganisms are involved in natural fermentation (Stanbury et al. 2017; Gomes et al. 2024). Arabica coffee, with its unique grape flavor profile, still needs to be explored in terms of isolation and identification of its microbial content. Previous research investigated using an inoculum containing the bacteria *Pediococcus acidilactici* and the yeast *Saccharomyces cerevisiae*. Still, it did not significantly improve coffee-cupping scores or wine-tasting notes (Rodrigues et al. 2020). Several studies have examined the effect of bacterial or yeast inoculum. These studies have primarily utilized an

approach that examines the alteration of volatile compounds (Evangelista et al. 2014; Ribeiro et al. 2017; Martins et al. 2019). Therefore, studies that focus on isolating and identifying microorganisms involved in natural coffee fermentation and assessing their enzymatic abilities, including pectinolytic, cellulolytic, proteolytic, and amylolytic activities, are critical to optimizing the fermentation process and eliciting wine flavor profiles.

This study aimed to identify beneficial bacterial and yeast strains to improve the coffee fermentation process and produce higher-quality coffee with a wine flavor taste. We isolated bacteria and yeast from natural fermentation, followed by enzymatic assays. DNA sequencing and phylogenetic analysis identified the bacteria and yeast with the highest enzymatic activity. Optimization experiments were conducted, and the resulting best ratio of bacteria-to-yeast inoculum consistently produced high-quality coffee with desirable wine characteristics, as evaluated by Q-Graders. This study highlights the potential application of microbial inoculants to enhance coffee quality and flavor.

MATERIALS AND METHODS

Natural coffee fermentation and isolation of bacteria and yeast

Ripe coffee cherries (*Coffea arabica* L. variety Line S) were sourced from Mount Puntang (1,400 meters above sea level) in West Java, Indonesia. The wine-coffee fermentation process involves storing 2 kg of coffee fruit in a tightly closed plastic container for 3 days and drying it in the sun for 15 days. After the cherries are dry, they are processed using a huller machine to produce green coffee beans.

Bacteria and yeast were collected during the four-day fermentation stage. One gram of coffee skin sample was diluted and placed in a petri dish with NA medium for bacteria and PDA medium for yeast. After 1-2 days of incubation, colonies were characterized, purified, and subjected to Gram staining and microscopic observation.

Isolate screening

Isolate screening involved observing enzymatic abilities, including pectinolytic, cellulolytic, amylolytic, and proteolytic activities. Transparent zones were monitored to assess their hydrolysis of pectin, cellulose, starch, and protein. Pectinolytic bacteria were screened using pectin Murashige and Skoog (MS) medium, containing yeast extract 1 $g.L^{-1}$, ammonium sulphate 2 $g.L^{-1}$, Na_2HPO_4 6 $g.L^{-1}$, KH_2PO_4 3 $g.L^{-1}$, citrus pectin 5 $g.L^{-1}$, and Agar 20 $g.L^{-1}$. Yeast screening used the Yeast Extract Peptone Dextrose (YEPD) containing peptone 20 $g.L^{-1}$, glucose 20 $g.L^{-1}$, yeast extract 10 $g.L^{-1}$, and agar 20 $g.L^{-1}$, added with 1% citrus pectin. Samples were incubated at 37°C for approximately 48 hours. After incubation, a 1% solution of Congo Red was applied for approximately 5 minutes, followed by rinsing with NaCl. The presence of a translucent zone indicated the occurrence of pectinolytic activity (Vidhyasagar et al. 2013).

Inoculating isolates screened cellulolytic bacteria to Carboxymethylcellulose (CMC) medium containing yeast extract 1 $g.L^{-1}$, CMC 5 $g.L^{-1}$, and agar 20 $g.L^{-1}$. At the same time, yeast screening was done using a medium with peptone 3 $g.L^{-1}$, yeast extract 3 $g.L^{-1}$, CMC 10 $g.L^{-1}$, and agar 20 $g.L^{-1}$. Samples were incubated at 37°C for approximately 48 hours. After incubation, a 1% Congo Red solution was added for about 5 min and then rinsed with NaCl. The translucent zone indicated cellulolytic activity (Jamroo et al. 2015).

Inoculating isolates screened amylolytic bacteria to Luria Bertani (LB) medium, containing yeast extract 5 g/L, NaCl 10 $g.L^{-1}$, and tryptone 10 $g.L^{-1}$ with 1% amylum. At the same time, yeast screening was done using a medium containing peptone 15 $g.L^{-1}$, yeast extract 10 $g.L^{-1}$, amylum 10 $g.L^{-1}$, and agar 20 $g.L^{-1}$. Samples were incubated at 37°C for approximately 48 hours. After incubation, a 1% Congo Red solution was added for about 5 min and then rinsed with NaCl. The translucent zone indicated amylolytic activity (Moradi et al. 2014).

The screening for proteolytic bacteria was conducted by inoculating isolates to LB agar medium, which contained yeast extract 5 g/L, NaCl 10 $g.L^{-1}$, and tryptone 10 $g.L^{-1}$, with 1% skim milk. At the same time, screening was done using a medium containing yeast extract 10 $g.L^{-1}$, peptone 15 $g.L^{-1}$, and agar 20 $g.L^{-1}$, with 1% skim milk. Samples were incubated at 37°C for approximately 48 hours. The translucent zone indicated proteolytic activity. The isolate with the highest qualitative activity score was selected for the wine-coffee fermentation process (Vijayaraghavan et al. 2013).

DNA sequencing and phylogenetic tree construction

Selected bacteria and yeast isolates from enzymatic assays were identified using DNA sequencing provided by Macrogen (http://dna.macrogen.com/eng/). For bacterial isolates, we targeted the 16S rRNA, and for yeast isolates, we targeted the ITS rRNA. Reference species were obtained using BLASTN (http://blast.ncbi.nlm.nih.gov/), and phylogenetic trees were constructed using MEGA 5 (Tamura et al. 2011).

Determination for the best of the age inoculum

Bacterial and yeast growth were monitored hourly to establish their growth curve. Bacterial cell count was assessed using the Total Plate Count (TPC) method, while a hemocytometer determined the yeast cell count. The cell counts, expressed as CFU/mL, were plotted on a logarithmic scale against time to generate growth curves. The optimal age of the inoculum was determined by identifying the point of maximum growth rate on the growth curve (Stanbury et al. 2017).

Coffee fermentation and processing

High-quality Arabica coffee cherries were chosen to produce wine coffee using solid-state fermentation. The cherries were washed and then fermented in enclosed plastic containers before drying. Different ratios of bacteria-to-yeast of 1:5, 1:10, and 1:20 were used, with total inoculum percentages of 5%, 10%, and 15% (v/w), respectively. Two kg of coffee fruit, supplemented with microbial inoculants, were placed in a tightly closed container, similar to the previously mentioned process.

Data was collected throughout the fermentation process up to the drying stage to monitor changes in the microorganism community, pH levels, and moisture content. Samples were taken on day 0 and day 1 of fermentation, as well as on days 5 and 10 during the drying process. TPC analysis was used to evaluate the dynamics of the microorganism community to evaluate the dynamics of the microorganism community. A 1-g coffee cherry sample was inoculated into a reaction tube with 9 mL of peptone water and diluted serially to obtain various dilution levels. 0.1 mL of each dilution was spread onto LB and YEPD agar medium and incubated at room temperature for 1-2 days. pH measurements were taken using a pH meter. A 1-g sample of coffee cherries was placed into a 9 mL reaction tube, and the pH level was measured (Madigan et al. 2019). For all analyses and graphics visualizations, R version 4.3.2 was used (R Core Development Team 2023).

After the fermentation process, the coffee cherries are dried in the sun for approximately ten days until the moisture content of the coffee beans reaches 12-13%. The cherries are then left for a week. After that, the outer layer of coffee skin is removed through hulling, leaving only the beans. The coffee beans are rested for two weeks before assessing the quality using the cupping test method.

Quality and sensory assessment

Two certified Q-Graders have conducted a cupping test. The test was carried out following the Specialty Coffee Association's prescribed methodology, which evaluates various parameters such as fragrance, flavor, aftertaste, acidity, body, balance, uniformity, cleanliness, sweetness, and overall score. Each parameter was rated on a scale from 0 to 10 points and then combined to determine the final cupping score. Based on the cupping test, coffee brewed with a total quality score of 80 or higher out of 100 is classified as specialty coffee (SCA 2015).

RESULTS AND DISCUSSION

Isolation and characterization of microorganisms

The process of natural fermentation in wine coffee involves various bacterial communities. During the study, we found 8 bacterial isolates in the fermentation and drying stages (Figure 1). Although this number is lower than that of a previous study that reported 11 bacterial isolates (Zhao et al. 2023), it is higher than the diversity of bacterial isolates in the Liberica coffee process in Indonesia, which only has five bacterial isolates (Wibowo et al. 2021). Three predominant strains were identified during the first three days of the fermentation stage: B1, B2, and B3. A previous study identified five bacterial isolates in fermentation (Krajangsang et al. 2022). Isolate B3 persisted during the drying stage on day 5, while only isolate B4 was detected during both drying stages on days 10 and 15. However, this study observed a lower bacterial community diversity than Krajangsang et al. (2022) study, where 9 bacterial isolates were reported during the drying stage.

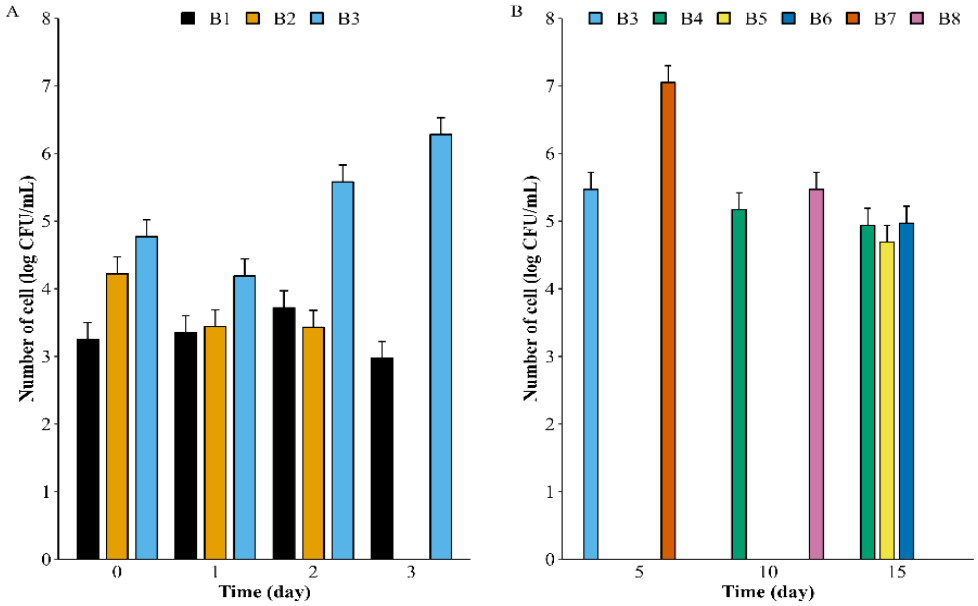

Figure 1. Dynamic population of bacteria communities in natural wine coffee fermentation ± SD. A. Fermentation stage; B. Drying stage. B1-B8 are bacteria isolates 1-8, respectively

The bacterial isolates were subjected to macroscopic and microscopic characterization, followed by culture purification to identify them. Gram staining of the bacterial isolates revealed four Gram-negative strains (B1, B3, B4, and B8) and four Gram-positive strains (B2, B5, B6, and B7). Gram-negative bacteria are commonly associated with pectin degradation from coffee fruit mucilage (Pereira et al. 2017). Circular colony shapes with flat borders were observed in all bacterial isolates except for B7 and B8, which displayed irregular borders. Isolates B1, B2, and B3 appeared transparent with pink, yellow, and white hues. Furthermore, isolates B2, B4, and B5 displayed cocci shapes, with others exhibiting coccobacilli morphology. This process of bacterial characterization serves as the preliminary screening process to identify the selected bacterial isolates (Cassimiro et al. 2023; Pino et al. 2023). These findings indicated that isolates B1, B2, and B3 were bacteria identified during the fermentation stages of the process for enzymatic analysis.

During the natural fermentation of wine coffee, the yeast community dynamics revealed the presence of five distinct yeast isolates across the fermentation and drying stages (Figure 2). This finding is not surprising since prior studies have also indicated a diversity of yeast isolates during coffee processing, ranging from four to nine yeast strains (Evangelista et al. 2014; Zhao et al. 2023). During the initial fermentation period (from day 0 to day 3), two predominant yeast isolates, Y1 and Y2, were identified. This result is lower than found in a previous study, which identified 4 to 8 yeast isolates during fermentation (Evangelista et al. 2014; Pereira et al. 2014). Although Y2 had the highest cell count, it was not detected on day 5 of the drying phase, while Y1 persisted until day 10 of the drying stage. Isolate Y3 was exclusively found on day 5 of the drying stage, while isolates Y4 and Y5 emerged solely on day 10. However, no yeast isolates were found on day 15 of the drying stage.

The yeast isolates were also characterized through macroscopic and microscopic examination. Macroscopic examination showed yeast colonies with circular shapes, white coloration, non-reflective surfaces, and flat borders, except for Y5, which had serrated borders. Microscopic examination revealed oval shapes for all isolates. Considering their similar macroscopic and microscopic characteristics, isolates Y1 and Y2 from the fermentation stage were chosen during pre-screening due to their highest cell count (Ribeiro et al. 2017; Martins et al. 2019).

Enzymatic assay

Bacterial isolates B1 and B3, as well as yeast isolates Y1 and Y2, were subjected to qualitative enzymatic assays, including tests for pectinolytic, cellulolytic, amylolytic, and proteolytic activities. The enzymatic tests on the most dominant coffee wine isolates revealed that all bacterial and yeast isolates exhibited pectinolytic activity (Table 1). Bacteria like *Klebsiella* sp., *Lactobacillus* sp., *Enterobacter* sp., and *Pantoea* sp. are known to produce pectinolytic enzymes in coffee fermentation (Abdollahzadeh et al. 2020; Pereira et al. 2020; Braga et al. 2023). Moreover, yeasts like *Saccharomyces* sp., *Pichia* sp., *Candida* sp., and *Torulaspora* sp. also produce pectinolytic enzymes in coffee fermentation, contributing to achieving the highest coffee scores (Braga et al. 2023; Ferreira et al. 2023).

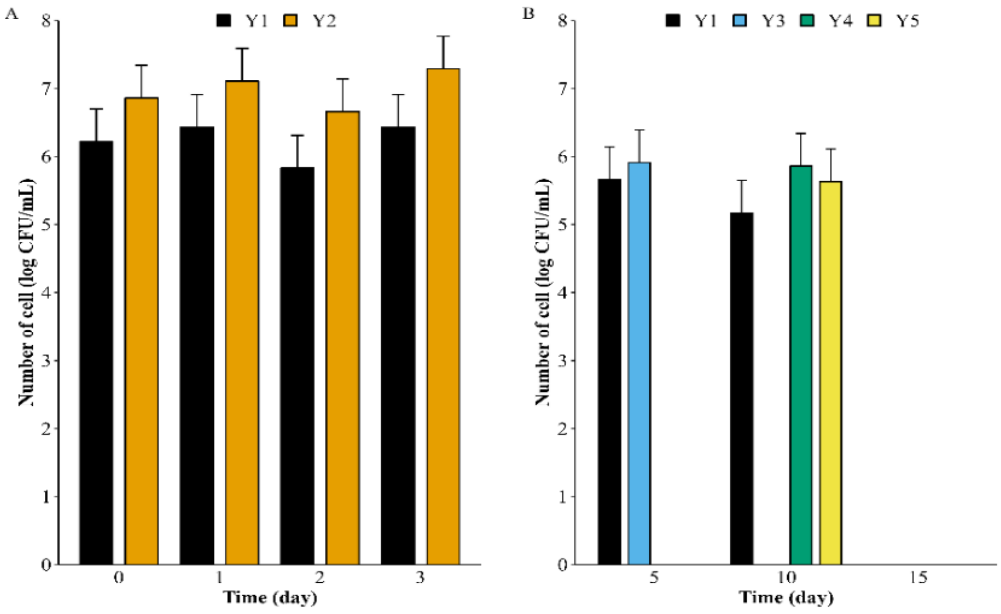

Figure 2. Dynamic population of yeast communities in natural wine coffee fermentation ± SD. A. Fermentation stage; B. Drying stage. Y1-Y5 are yeast isolates 1-5, respectively

The cellulolytic activity was found only in the B3 bacterial isolates, indicating their ability to convert sucrose into glucose and fructose enzymatically (Pereira et al. 2020). On the other hand, only the yeast isolates exhibited amylolytic activity, indicating the hydrolysis of starch, as evidenced by the appearance of a red color (Vidhyasagar et al. 2013; Moradi et al. 2014). Additionally, bacterial isolate B1 and yeast isolates Y1 and Y2 showed proteolytic activity. This process involves the proteolytic breakdown of long-chain peptides into shorter amino acid chains (Stanbury et al. 2017; Pereira et al. 2020). However, the specific bacterial and yeast species responsible for these enzymatic processes during coffee fermentation remain unknown in current literature.

Phylogenetic tree construction

Based on the result mentioned above, it was found that bacterial isolate B3 and yeast isolate Y1 were the most effective and could be used as fermentation inoculum. To further identify them, we performed DNA sequencing and created a phylogenetic tree with bootstrap values on the tree branches. These values indicate the reliability of the tree construction, with higher values suggesting more robust support for relationships between species (Soltis and Soltis 2003). Based on the phylogenetic tree shown in Figure 3, B3 was identified as a member of the *Klebsiella* genus. Although the evolutionary distance from *Klebsiella quasipneumoniae* was close to 0, the bootstrap value fell below 90%, indicating that further investigation is required. *Klebsiella* is a genus of nonmotile, Gram-negative, rod-shaped bacteria that can ferment lactose (Vidhyasagar et al. 2013). It belongs to the Enterobacteriaceae family, exhibits facultative anaerobic metabolism, and can utilize citric acid and glucose as carbon sources and ammonia as the sole nitrogen source. Several studies have reported that common bacteria in coffee with pectinolytic activity include genera such as *Klebsiella, Erwinia, Aerobacter, Escherichia*, and *Bacillus* (Abdollahzadeh et al. 2020; Pereira et al. 2020; Braga et al. 2023). It is important to note that species within the Enterobacteriaceae family are often considered opportunistic pathogens that may contaminate coffee during cultivation or fermentation, especially on the surface of coffee cherries, potentially influencing mucilage decomposition. The decomposition process of mucilage is primarily carried out by Gram-negative, lactose-fermenting bacteria originating from soil (Abdollahzadeh et al. 2020).

Table 1. Predominant enzymatic microorganisms in wine coffee fermentation process

Sample	Isolate	Pectinolytic	Cellulolytic	Amylolytic	Proteolytic
Bacteria	B1	++	-	-	+
	B2	++	-	-	+
	B3	++	++	-	-
Yeast	Y1	+	-	+	+
	Y2	+	-	+	+

Note: (+): There is clear zone (more + signs represent a larger clear zone); (-): There is no clear zone

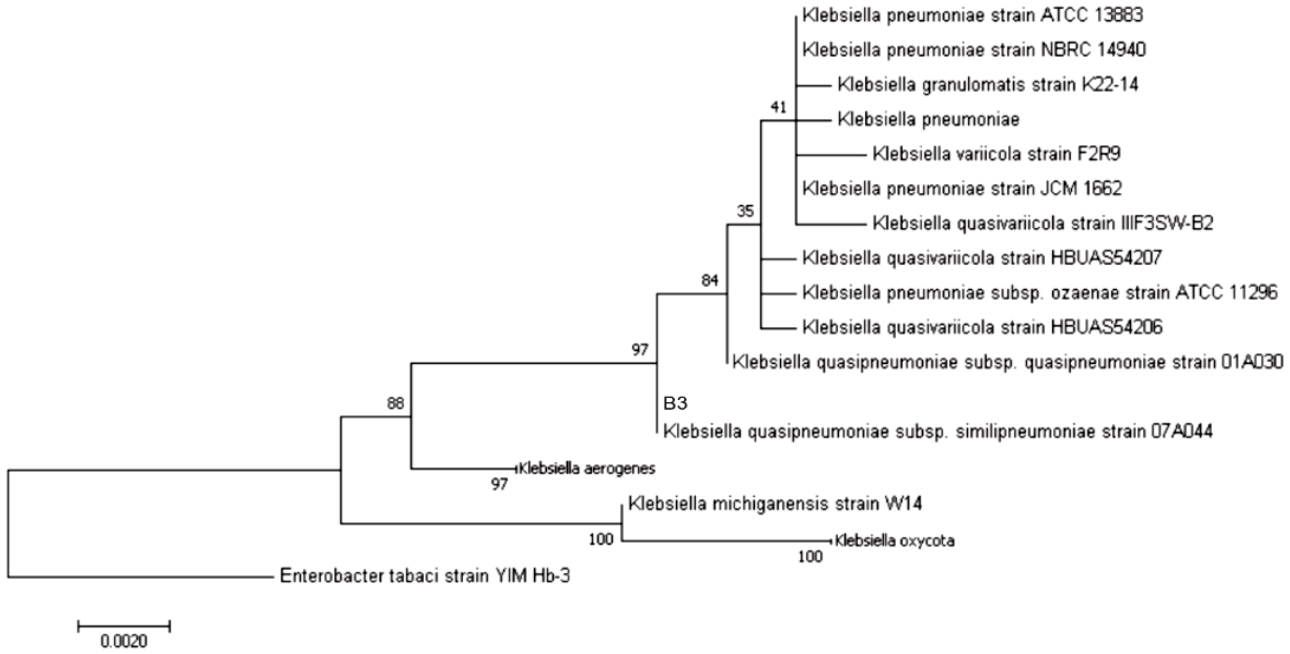

Figure 3. Phylogenetic tree of bacteria isolate B3

The Y1 yeast strain was identified as *Pichia kudriavzevii* through a phylogenetic tree (Figure 4). This elongated oval-shaped yeast can be found in soil, fruit skins, and various fermentation processes (Bressani et al. 2021). It can remain active at temperatures up to 45°C and pH 2, producing ethanol at a high rate. *P. kudriavzevii* is a common yeast species used in the fermentation of wine, beer, and cocoa beans (Oberoi et al. 2012; Schwan et al. 2023; Chang et al. 2024). Other yeast species like *P. kluyveri, P. anomala, S. cerevisiae, Hanseniaspora uvarum, Debaryomyces hansenii*, and *Torulaspora delbrueckii* are also present in coffee (Braga et al. 2023; Schwan et al. 2023; Gomes et al. 2024).

Starter and preparation

Determining the best inoculum age requires analyzing the growth rate of microorganisms, with a focus on the logarithmic phase of the growth curve. This phase shows the microbe's peak physiological state and cell replication rate, making it ideal for use as inoculum (Stanbury et al. 2017; Madigan et al. 2019). The responses of *Klebsiella* sp. and *P. kudriavzevii* to changes in pH varied significantly. The growth curve of *Klebsiella* sp. was monitored hourly (Figure 5.A), and its logarithmic phase occurred between 0 and 8 hours, followed by the stationary phase. The pH remained stable during the logarithmic phase until the 5th hour, thanks to bicarbonate production by *Klebsiella* sp. The pH increased as the bacteria entered the stationary phase due to cell death and lysis, which released intracellular proteins and essential compounds into the medium, elevating its pH (Elhalis et al. 2023). Based on the growth curve analysis, *Klebsiella* sp.'s optimal inoculum age was 4 hours, with a specific growth rate of 0.033 per hour and a generation time of 39.7 minutes.

In the growth curve of *P. kudriavzevii*, monitoring occurred at intervals of two hours (Figure 5.B). The logarithmic phase of *P. kudriavzevii* lasted from 0 to 12 hours, followed by the stationary phase. During the logarithmic phase, the medium's pH decreased gradually.

This decline was attributed to the yeast's production of various carbonic compounds and organic acids during carbon metabolism, which were released from the cells to maintain intracellular pH homeostasis (Krajangsang et al. 2022). Conversely, during the stationary phase, there was a subsequent increase in pH within the medium. This increase resulted from yeast adaptation to nutrient depletion, producing ammonia to alkalize the medium, facilitating yeast survival under stress conditions, albeit with reduced growth rates and altered physiological states (Chang et al. 2024). Based on the growth curve analysis, it was determined that the optimal inoculum age for *P. kudriavzevii* was at 6 hours, exhibiting a specific growth rate of 0.014 per hour and a generation time of 72.6 minutes.

Dynamic of microorganism community and pH value during the fermentation

The community dynamics of microorganisms were analyzed to optimize wine coffee fermentation, using three different ratios of bacteria *Klebsiella* sp. to yeast *P. kudriavzevii*: 1:5, 1:10, and 1:20. Figure 6 shows the results of each ratio and the inoculum percentages of 5%, 10%, and 15% (v/w), along with control in both fermentation and drying stages. In summary, introducing bacteria and yeast significantly increased their count compared to the control after one day of fermentation across all inoculum ratios and percentages. This result indicates that the introduced microorganisms potentially outcompeted the naturally occurring microorganisms, which is crucial in driving the coffee fermentation process (Evangelista et al. 2014). The total bacterial cell count was lower than that of yeast on day 0, but after one day of fermentation, the bacterial count surpassed that of yeast. This shift can be attributed to the faster generation time of *Klebsiella* sp. compared to *P. kudriavzevii*. It's a common observation that bacteria generally have a shorter generation time than yeast (Pei and Schmidt 2018; Madigan et al. 2019; Cassimiro et al. 2023).

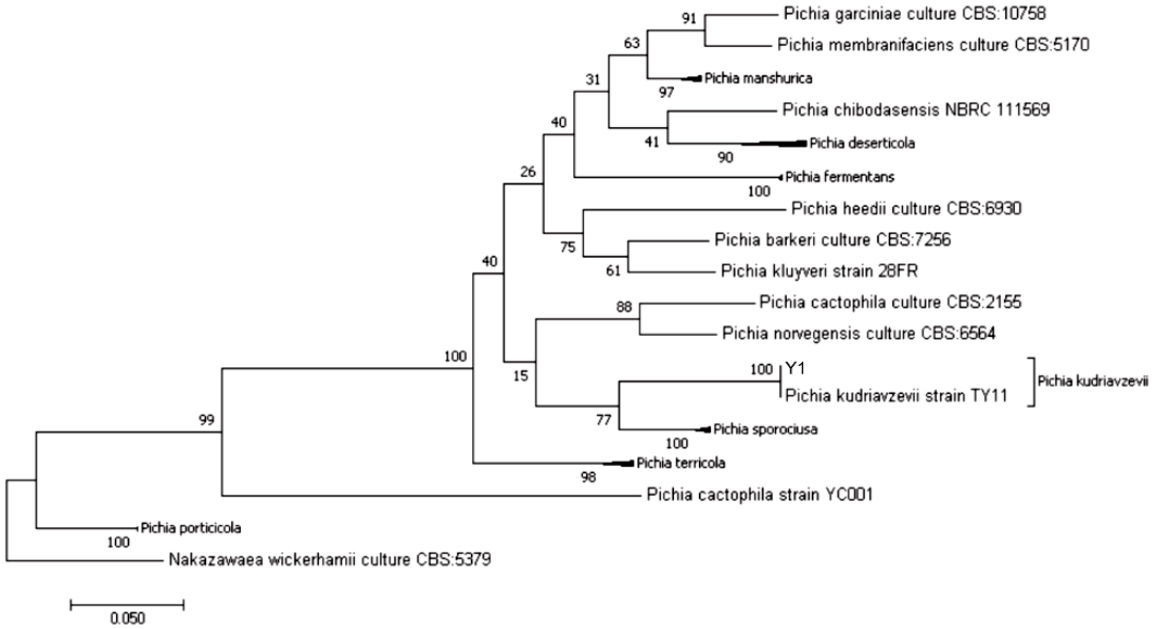

Figure 4. Phylogenetic tree of bacteria isolate Y1

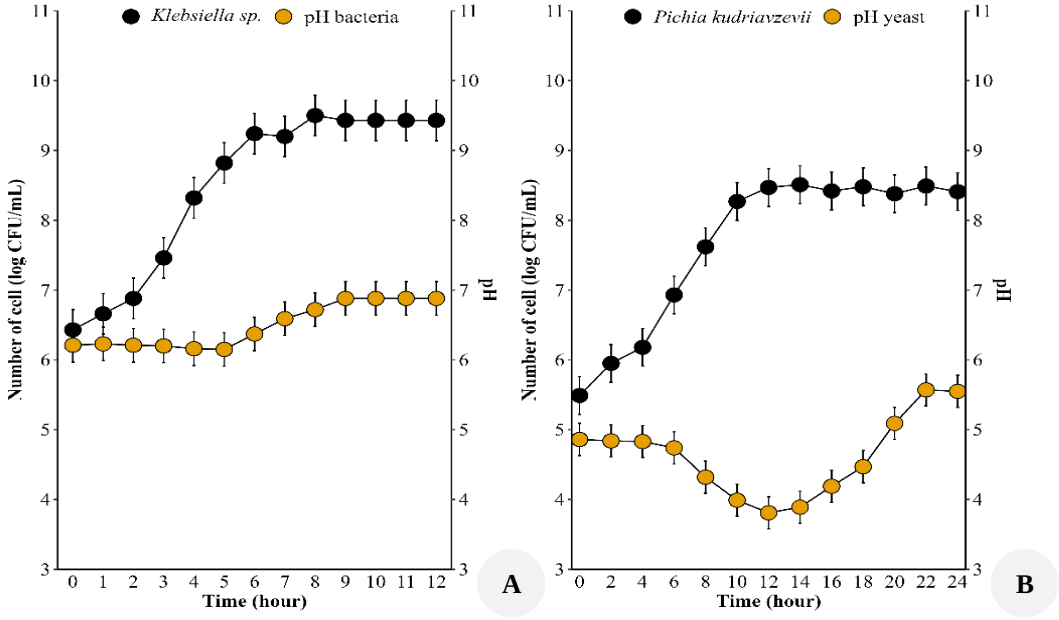

Figure 5. The growth curve of bacteria and yeast with pH condition. A. Bacteria *Klebsiella* sp.; B. Yeast *Pichia kudriavzevii* Data are means ± SD

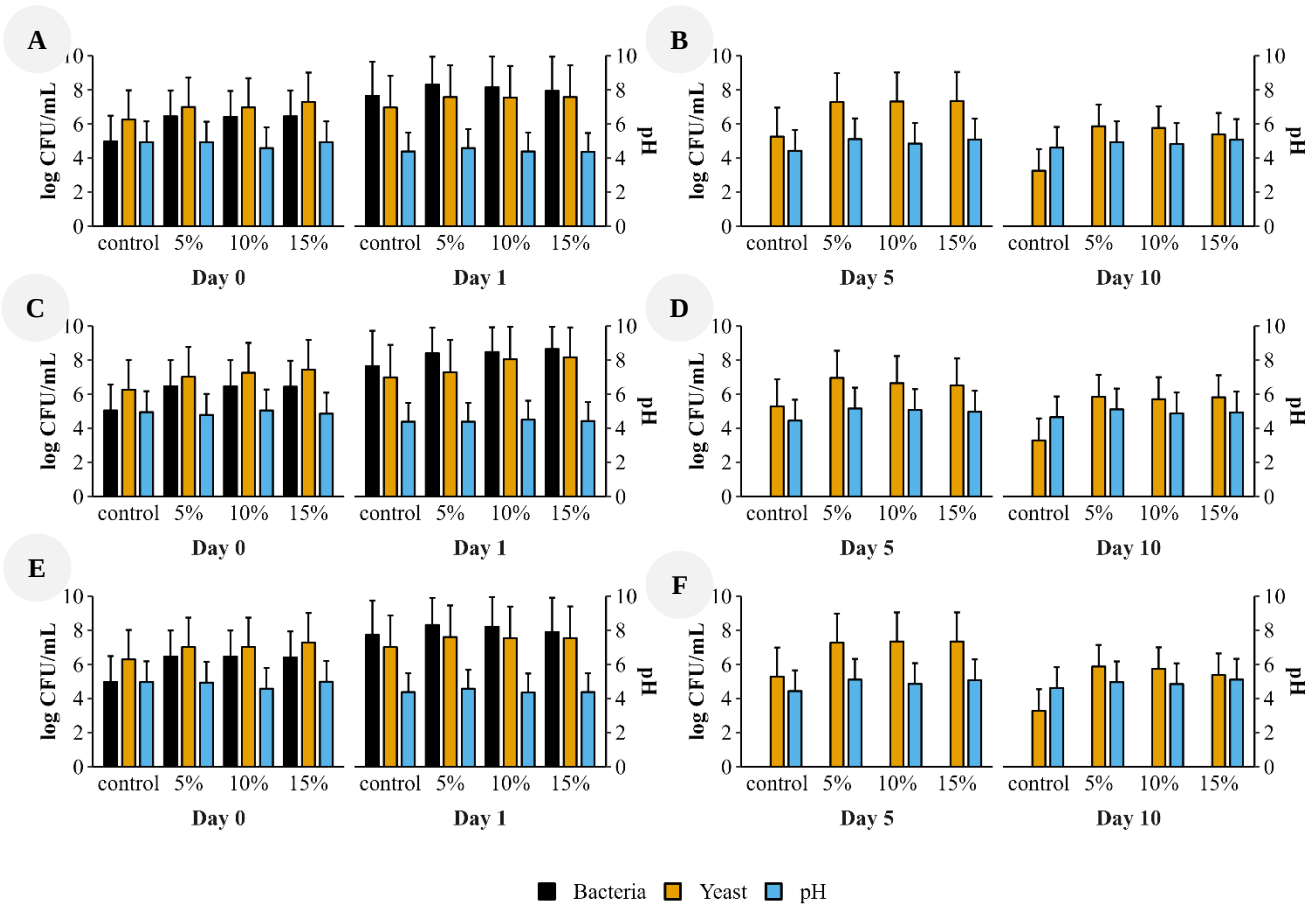

Figure 6. The dynamic population of bacteria and yeast ratio with pH conditions in different stages. A. Ratio 1:5 in fermentation stage; B. Ratio 1:5 in drying stage; C. Ratio 1:10 in fermentation stage; D. Ratio 1:5 in drying stage; E. Ratio 1:20 in fermentation stage; F. Ratio 1:20 in drying stage. Data are means ± SD

During the drying process on the fifth and tenth days, only *P. kudriavzevii* was detected, with no presence of *Klebsiella* sp.. This contrasted with the fifth day of the isolation process, where *Klebsiella* sp. persisted. The survival of *Klebsiella* sp. during this stage is attributed to suboptimal light-intensity conditions, which allowed for its survival. The microbial numbers decrease during drying due to water evaporation, primarily driven by sunlight exposure (Rocha et al. 2023). *P. kudriavzevii* is more tolerant of low water content and pH than bacteria, so it persists during drying (Oberoi et al. 2012; Krajangsang et al. 2022). The pH values showed a similar pattern across all variables, with an initial pH of around 5.0 during fermentation decreasing to 4.0-4.5, followed by an increase to around pH 5.0 during drying. Control samples showed more stable pH values post-fermentation and during drying, indicating lower metabolic activity due to reduced bacterial and yeast abundance. The decrease in pH is due to the production of organic acids like lactic acid and ethanol during fermentation. In contrast, the increase in pH is associated with using organic acids as carbon sources by yeast (Vaz et al. 2023). The evaporation of volatile acids during drying may also influence the pH dynamics (Zhao et al. 2023).

Cupping test

The Specialty Coffee Association of America (SCAA) developed the cupping test in 2015 to provide a structured approach to assessing coffee quality. This evaluation involves certified panelists examining coffee from its green bean stage through the roasted phase (SCA 2015). The final cupping test achieved the highest score when 15% (v/w) inoculum was added, with a bacteria-to-yeast ratio of 1:10 (Table 2). Tasting notes include chocolate, ripe cherry, and tannin. Tannin compounds in grape skins contribute to a tannic flavor reminiscent of red wine. The cupping test results showed a positive correlation between higher inoculum additions and increased cupping scores, attributed to elevated microbial cell counts and substrate availability during fermentation (Rodrigues et al. 2020).

Coffee cherries, especially their mucilage, are rich in complex carbohydrates that microorganisms convert into precursor compounds, shaping the coffee's taste profile. The coffee has unique flavors such as fruity, acidic, and wine flavor, similar to red wine (Pereira et al. 2014). The winey fragrance, which is only prominent when 15% inoculum is added, results from increased diacetyl and acetoin formation during coffee fermentation. Yeast inoculation enhances sensory attributes, particularly fruity, buttery, and fermented fragrances. The winey flavor in coffee comes from precursor flavors such as diacetyl and acetoin. Diacetyl, a byproduct of amino acid metabolism, is formed through alpha-acetolactate oxidative decarboxylation during yeast exponential growth. Diacetyl is then enzymatically reduced to acetoin by diacetyl reductase (Pereira et al. 2016; Ferreira et al. 2023).

Lower yeast abundance can decrease taste components in a 1:5 ratio of yeast to coffee. On the other hand, in a 1:20 ratio, excessive yeast presence can accumulate metabolic byproducts, contributing to a bitter, over-fermented flavor. Coffee flavor is predominantly developed during roasting, which involves complex reactions such as the Maillard reaction and caramelization. These reactions alter the flavor precursors of green coffee beans. Around 300 volatile compounds contribute to coffee flavor, including 2-furfurylthiol, 4-vinyl guaiacol, and various alkyl pyrazines formed during roasting. Ethanol, acetaldehyde, ethyl acetate, and isoamyl acetate from yeast metabolism, along with hexanal and 2,3-butanedione from fermentation thermal reactions, also play a significant role in flavor development (Evangelista et al. 2014; da Silva Vale et al. 2019).

Arabica coffee is known to have a higher cupping score when inoculated with *Klebsiella* sp. and *P. kudriavzevii*, compared to when inoculated with *Pediococcus acidilactici* and *S. cerevisiae* in previous studies (Rodrigues et al. 2020). This inoculation process also causes the appearance of a wine aroma in the coffee. These findings suggest that the inoculum used in this study is more effective in enhancing coffee quality. The trials leading to these findings were conducted with a small amount of coffee, and scaling up of fermentation should take into account the preparation of the active microbes for inoculation. In Indonesia, Arabica coffee consistently gets higher cupping scores than Robusta and Liberica coffee, which has gained widespread popularity (Wibowo et al. 2021; Fauzi et al. 2023). This inoculation method is likely to be applied to Robusta and Liberica coffee, with some consideration for slightly different mucilage characteristics and the dominant flavors already present in the other species.

Table 2. Cupping scores from optimization wine coffee fermentation

Inoculum		Score		Average	Result	Rank
Ratio	Percentage	Q grader 1	Q grader 2			
Control	-	81.75	81.50	81.62	Specialty coffee	8
1:5	5%	80.00	83.25	81.63	Specialty coffee	7
	10%	81.00	83.25	82.13	Specialty coffee	5
	15%	81.75	85.00	83.38	Specialty coffee	4
1:10	5%	78.25	78.50	78.38	Not specialty coffee	10
	10%	83.50	83.75	83.63	Specialty coffee	2
	15%	84.75	83.75	84.25	Specialty coffee	1
1:20	5%	81.75	82.00	81.88	Specialty coffee	6
	10%	83.00	79.75	81.38	Specialty coffee	9
	15%	82.75	84.50	83.62	Specialty coffee	3

In conlusion, we discovered three bacterial and two yeast isolates during the natural coffee fermentation process. Two isolates, *Klebsiella* sp. and *P. kudriavzevii*, showed potential as inoculants. Using the two isolate ratios, the bacteria-to-yeast ratio was 1:10 with an inoculum of 15% (v/w) consisting of *Klebsiella* sp. and *P. kudriavzevii*, producing specialty coffee of the highest quality. This inoculation process also gave the coffee a wine flavor characteristic compared to the natural process. Therefore, inoculating Arabica coffee fermentation with *Klebsiella* sp. and *P. kudriavzevii* can significantly enhance the coffee's quality, notably enriching it with the esteemed wine flavor profile.

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