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DYNAMICS COMMUNITIES OF BACTERIA AND YEAST IN PROCESSING ROBUSTA COFFEE WITH WET PROCESSING: SCREENING OF DOMINANT ISOLATES

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ABSTRACT

The type of coffee processing has a direct impact on the microbial community it contains and the quality of the coffee it produces. Coffee processing with a wet process (wet) is known to produce coffee with a good aroma and taste profile. This study aims to determine the dynamics of the bacterial and yeast community during the postharvest process and to determine the dominant microbes involved in the processing of Robusta coffee using a wet process. The method used was the isolation of bacteria and yeast using selective medium with the addition of antibiotics, then morphological identification and calculation of the index of diversity (H), dominance (D) and evenness (E) were carried out based on the bacterial and yeast isolates obtained, as well as screening of dominant isolates based on results of dominance analysis and enzymatic activity. The isolation results obtained by the yeast community (51.7%) with a higher number than the bacterial community (48.3%). The microbial community in the wet processing of Robusta coffee had a species diversity index (H') = 2.48, evenness (E) = 0.74 and dominance (D) = 0.17. The results of isolates screening through enzymatic tests showed bacterial isolates B10 and yeast Y2 were selected as dominant isolates and could be used as starter cultures in a controlled fermentation process.

Keywords : *dynamics, community, robusta coffee, wet process*

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INTRODUCTION

Postharvest coffee processing is a series of long processes and is known to affect the quality of the coffee drink produced. Based on the process, coffee processing can be done in 3 ways, namely wet, semi-dry and dry. Wet processing is processing that involves the use of water where the picked coffee cherries are then peeled and fermented by soaking them in a container filled with water for a certain length of time then washed and dried. Semi-dry processing is a transitional process between dry and wet processing in which the peeled coffee beans are then dried. Dry processing is also known as natural processing, where the picked coffee cherries are then dried without going through a fermentation process under water. Coffee processing with wet processing (wet) is known to have advantages in the quality of the resulting aroma. The stages in wet processing are picking, water sorting, pulping, fermentation, washing, drying, hulling, resting, roasting, grinding and storage (Nusaly, 2020).

During the coffee postharvest process starting from the picking stage to drying it is inseparable from the activities of various microbial communities, even during coffee fermentation, microbes produce various metabolites. Microbial activity during fermentation determines the concentration of free sugars (glucose and fructose) and free amino acids in coffee beans and further contributes to the production of Maillard compounds and volatiles during the roasting process. The impact of fermentation on wet processing is known to improve coffee quality due to the presence of aromatic compounds produced during degradation of the mucilage layer by microbial activity (Haile, 2019). In addition to having a positive impact, an uncontrolled fermentation process can cause overflection which can produce coffee with an unwanted taste.

Previous research that has been done previously shows that with control during fermentation through the addition of dominant microbes and controlled fermentation time it is able to produce coffee with fine quality (Syahchnormalietea, 2018). By knowing the profile of the microbial community and its role during the wet processing of coffee, it can be determined which microbes play a dominant role in these stages. The selection of suitable microbes through the dominant isolate screening process is considered important to do, because it has a positive impact on the taste and aroma of coffee during fermentation. The dominant microbes obtained can be used as starter cultures and are expected to be commercialized for use in the coffee processing industry in a controlled manner.

METHOD

1. Isolation of Bacteria and Yeast

The medium used in the microbial isolation stage was NA (Nutrient Agar) added with the antibiotic Nystatin for bacterial isolation, PDA (Potato Dextrose Agar) added with the antibiotic Amoxcylin for yeast isolation and 0.89% NaCl as a physiological solution. For isolation of lactic acid bacteria (LAB) using selective medium MRS agar. Robusta coffee samples were taken from coffee plantations in the Lampung area, Kab. West Lampung with an altitude reaching 848 meters above sea level. Sampling was carried out at each post-harvest stage of wet processing starting from picking (P1), water sorting (P2), pulping (P3), fermentation (F), washing (AW) and drying (48-240 hours). In the drying stage, sampling is carried out over an interval of 2 days (48 hours) until the moisture content reaches 1113%. Each sampling was measured pH, temperature and water content. Microbe isolation using the spread plate method and incubated at room temperature for 1-3 days. All growing colonies were coded isolate and TPC (Total Plate Count) was calculated (Capucino, 2008):

$$TPC = \frac{\text{Number of colonies}}{\text{Dilution level}} \times 10$$

Microbial purification using the quadrant scratch method. Gram test using the KOH method [15]. Isolate preservation was carried out in slanted agar media and glycerol stock [4].

2. Determination of Diversity Index (H), Dominance (D) and Evenness (E)

Each bacterial and yeast isolate obtained from the entire postharvest stage was then identified by morphology and given an isolate code, then analyzed for community indices (Species Diversity (H'), Dominance (D) and Evenness (E) (Capucino, 2008).

Species Diversity Index Formula (Shanon-Wiener) (Sulistyani, 2014 namely:

$ID = H' = -\Sigma Pi \ln$ dimana $= \frac{ni}{N}$

Information:

ni = number of individuals of each species

N = total number

H' = Shanon-Winner diversity index

Pi = abundance index

3. Enzymatic Activity

Testing of pectinolytic activity in bacteria using LB medium (Luria Bertani) agar containing 5 g L-1 citrus pectin (Widowati et al, 2014) and in yeast using Yeast Extract Peptone Dextrose Agar (YEPDA) medium containing 10 g L-1 citrus pectin (Barutu, 2017). The test solution uses 5 mL Congo red 1%. Testing for amylolytic activity in bacteria used LB medium (Luria Bertani) agar containing 10 g L-1 starch (Moradi et al, 2014) and in yeast using Yeast Extract Peptone Dextrose Agar (YEPDA) medium containing 10 g L-1 starch (Wulandari, 2017). The test solution uses 5 mL of Lugols solution. Testing cellulolytic activity in bacteria using LB medium (Luria Bertani) agar containing CMC (Carboxy Methyl Cellulose) 5 g L-1 (Hidayat, 2004) and in yeast using Yeast Extract Peptone Dextrose Agar (YEPDA) medium containing 10 g L-1 CMC (Carasco et al, 2016). The test solution uses 5 mL Congo red 1%. Testing of proteolytic activity in bacteria using LB medium (Luria Bertani) agar containing 20 g L-1 skim milk (Novalia, 2014) and in yeast using Yeast Extract Peptone Dextrose Agar (YEPDA) medium containing 28 g L-1 skim milk (Rodarte et al, 2011). Bacterial isolates aged 24 hours and yeast aged 48 hours were transferred to selective medium, then incubated at 37 oC for 24-48 hours. A positive reaction is indicated by the presence of a clear zone around the colony.

4. Screening of Dominant Isolates

Isolate screening was carried out to obtain dominant bacterial and yeast isolates which would be used as starter cultures. This was done based on the highest dominance value obtained from each isolate and its ability to hydrolyze substrates (possess enzymatic activity).

DISCUSSION RESULT

In wet processing of Robusta coffee with samples of coffee cherries from coffee plantations in West Lampung district, a bacterial community was obtained with a percentage of 48.3% (14 isolates). The results of the gram test showed that of the 14 isolates of bacteria that were successfully isolated, 71.4% were gram-positive bacteria (10 isolates namely B1, B5, B7, B8, B10, B11, B13, B14, B15 and B18) and 28.6% is a gram-negative bacteria (4 isolates namely B2, B3, B9 and B12).

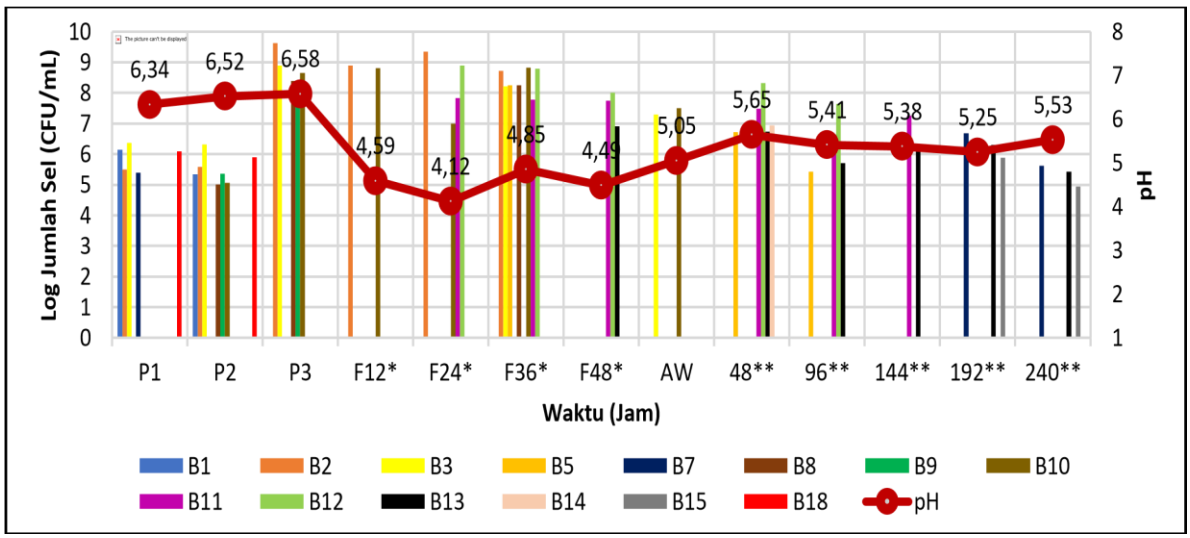

Figure 1. Dynamics of Bacterial Community in Wet Processing of Robusta Coffee
(Remarks: B = Bacterial Isolate; P1 = Plucking; P2 = Sorting water; P3 = Pulping; F (*) = Fermentation, AW=After Washing, 48-240 (**) = Drying).

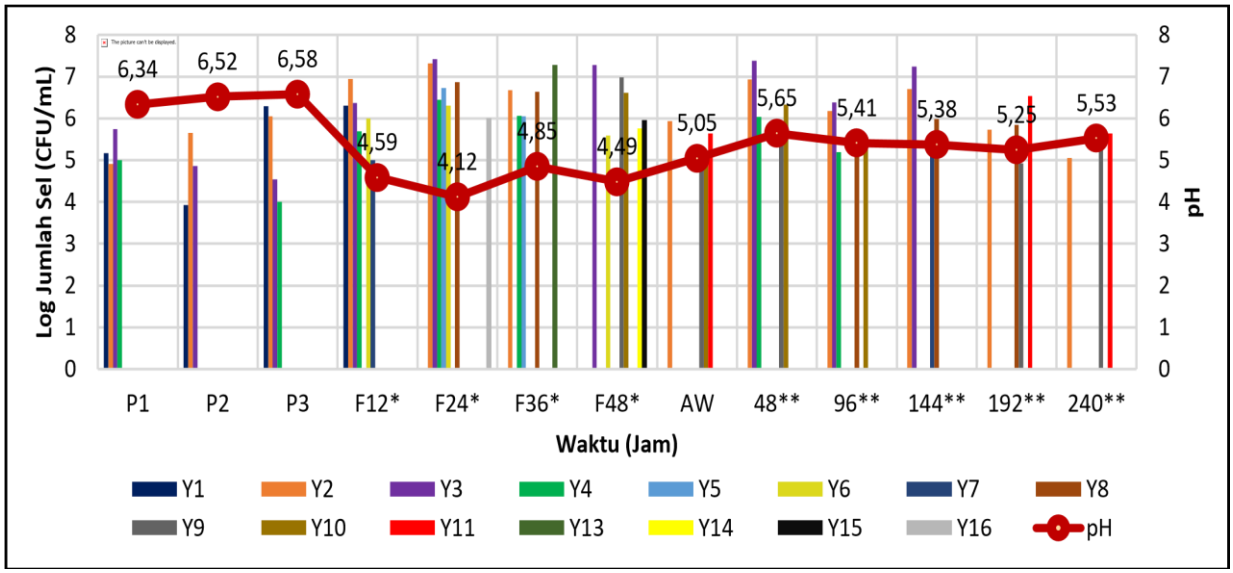

Figure 2. Dynamics of Yeast Community in Wet Processing of Robusta Coffee (Remarks: Y = Yeast Isolate; P1 = Picking; P2 = Water sorting; P3 = Pulping; F (*) = Fermentation, AW = After Washing, 48-240 (**) = Drying).

14 bacterial isolates that were successfully isolated, 4 of them were lactic acid bacteria, namely isolates B7, B8, B14 and B15. The bacterial community at the picking stage (P1) was 6.8 log CFU/mL and reached the highest community at the pulping stage (P3) with a total population of 9.8 log CFU/mL (Figure 1). In addition to the bacterial community, in Robusta coffee processing with wet processing, a yeast community was found with a higher percentage than bacteria, namely 51.7% (15 isolates namely Y1, Y2, Y3, Y4, Y5, Y6, Y7, Y8, Y9, Y10, Y11, Y13, Y14, Y15 and Y16). The yeast community present at the picking stage (P1) was 5.9 log CFU/mL and reached the highest community in 24 hour fermentation (F24) with a total of 7.8 log CFU/mL
The results of the analysis of community indices showed that the index of diversity of types of bacteria and yeast in Robusta coffee processing with wet processing (wet) was classified as very stable at the 36-hour fermentation stage with a value of H' = 2.48 (Table 1). The higher the H' value, the higher the stability of the bacterial and yeast community in the wet processing of Robusta coffee. A community that has an H' value < 1 is said to be a less stable community, if the H' value is between 1-2 it is said to be a stable community, and if the H' value > 2 is said to be a very stable community (Mawazin, 2013). The high H' value at the 36-hour fermentation stage (F36) was due to the high water content and high nutrients during fermentation which facilitated microbial growth so that at this stage there were abundant types of microbes, as well as more than the other stages. To determine the level of stability of a species in a community, the value of E is used (E = 0 < 0.3 the level of stability of species diversity is low; E = 0.3 <0.6 the level of stability of species diversity is classified as medium; E = > 0.6 level the stability of species diversity is high [8], Table 1 shows that the 36-hour fermentation stage (F36) has the highest E value, reaching 0.74 and the lowest E value is found in the after-washing (AW) stage of 0.52. At this stage, most Most of the bacteria and yeast were lost due to washing after fermentation.At stage F36 it was known that the community was classified as having a high level of stability of diversity, while at stage AW it was known to have a level of stability of diversity which was classified as moderate.

The results of the species dominance index analysis showed that at stages P1, P2 and F24, bacterial isolates B3 were dominated with D values at each stage, namely 0.015, 0.014 and 0.013. At stages P3 and F12 were dominated by bacterial isolates B2 with D values at each stage of 0.022 and 0.027. The F36 and AW stages were dominated by bacterial isolate B10 with D values of 0.009 and 0.042. At stage F48, 48-96 hours of drying, the bacterial isolate B12 was dominated with D values of 0.017, 0.014 and 0.019, respectively. After 144 hours of drying it was dominated by bacterial isolate B11 with a D value of 0.035. After 192 hours of drying it was dominated by bacterial isolate B7 with a D value of 0.025. At the end of drying it was dominated by yeast Y11 isolate with a D value reaching 0.030. The results of the analysis of species dominance as a whole showed that the highest species dominance was obtained by bacterial isolates B10 (D = 0.042) and yeast Y2 (0.026) at the AW stage. The high dominance of the two isolates above indicates a concentration of dominance only on certain species, so that the dominance index at the AW stage becomes high (D = 0.17)

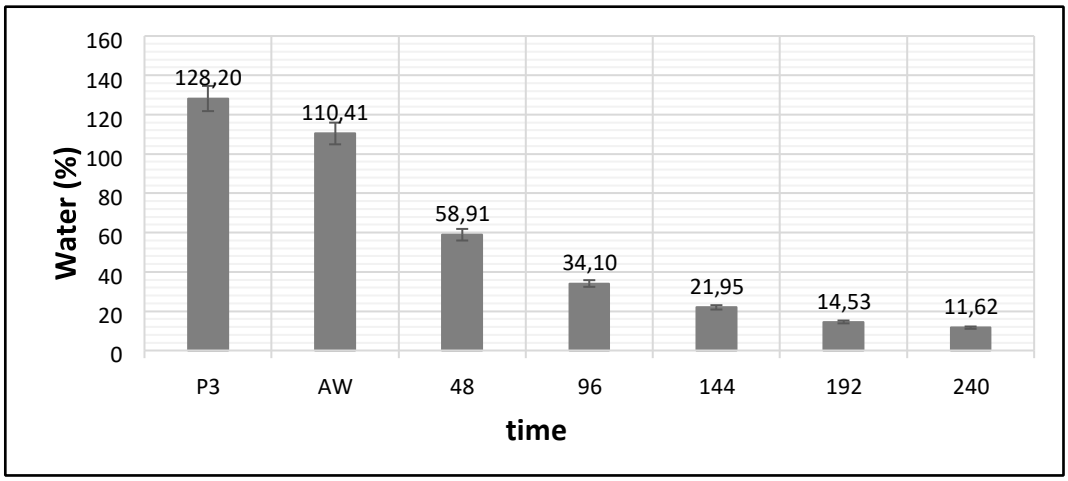

Figure 3. Percentage of Water Content (%) of Robusta Coffee Beans During the Drying Stage with Wet Processing (Remarks: P3 = Pulping; AW = After Washing; 48-240 hours = Drying).

The high dominance index indicates that the abundance of each species in this area is not evenly distributed, so the evenness index is lower (E = 0.52) (Table 1). These results are in accordance with the opinion of a researcher who states that the dominance of certain species and the uneven distribution of species causes the value of evenness to decrease [7]. The low evenness and low number of these species causes low diversity in this area (H'=1.78)

Table 1. Number of Species, Number of Individuals, Diversity Index, Dominance Index and Evenness Index.

	P1	P2	P3	F12	F24	F36	F48	AW	48	96	144	192	240
S	9	10	9	8	11	12	9	6	10	9	6	7	6
N	50,30	52,99	64,63	54,01	80,14	91,53*	60,82	36,40	68,78	54,26	38,60	41,92	32,25
H'	2,19	2,30	2,16	2,06	2,39	2,48*	2,19	1,78	2,30	2,19	1,79	1,94	1,79
D	0,11	0,10	0,12	0,13	0,09	0,08	0,11	0,17*	0,10	0,11	0,17	0,14	0,17
E	0,65	0,68	0,64	0,61	0,71	0,74*	0,65	0,52	0,68	0,65	0,53	0,58	0,53

Description: S = Number of Types; N = Number of Individuals; H' = Diversity Index; D = Dominance Index; E = Evenness Index; * = Highest Value.

At the beginning of processing up to 96 hours after drying, a higher number of bacterial communities was found compared to the yeast community. This is because bacteria have a shorter generation time than yeast and the ability to consume simple sugars as a carbon source, besides that several types of bacteria are known to have the ability to hydrolyze pectin to produce simple sugars (glucose, ramnose, L-arabinose and D-galacturonic acid).) as an additional carbon source for yeast metabolism (Pereira et al, 2018). The number of bacterial communities decreased when entering the washing and drying stages. The washing process is thought to remove the remaining slime layer attached to the coffee bean parchment while during the drying process the water content decreases gradually. This condition certainly causes drought stress, the loss of most of the nutrient content and the buildup of metabolic products in the form of organic acids have a direct impact on the decrease in the number of bacterial communities. At the end of drying, i.e. at 144 hours to 240 hours of drying, a yeast community was found with a higher number than the bacterial community. This is because yeast has the ability to survive drought stress conditions during the drying process by metabolizing simple sugars which are the result of bacterial metabolism at the start of processing (Pereira et al, 2014).

The dynamics of the bacterial and yeast communities during the coffee processing process are related to the content of polysaccharides (pectin, cellulose and starch), simple sugars and proteins found in coffee beans which are a source of nutrition for microbial growth. The activity of pectinolytic, cellulolytic, amylolytic and proteolytic enzymes produced by microbes contributes to the hydrolysis of simple sugars, polysaccharides (pectin) and proteins found in the skin, pulp and mucilage of coffee. At the P1 (picking), P2 (water sorting) and P3 (pulping) stages, pectinolytic, amylolytic, proteolytic and cellulolytic microbial communities were found with the highest number of communities present at the P3 stage. This is because in the mesocarp layer (flesh and mucilage) coffee fruit is rich in carbohydrates (glucose, fructose and pectin) protein, fat, minerals, tannins, polyphenols and caffeine. Pectin is the main carbohydrate polymer found in the mesocarp and at the P3 stage it

can be seen that the highest bacterial community comes from pectinolytic microbes. Hydrolysis of pectin produces simple sugars (glucose, ramnose, L-arabinose and D-galacturonic) as a carbon source for yeast metabolism and as precursors in aroma formation. The cellulolytic microbial community was also present during the P1, P2 and P3 stages with the highest activity at the P3 stage, because the endocarp layer is known to be rich in cellulose, hemicellulose, lignin and ash. Amylolytic microbes were found in the early stages (P1, P2 and P3) as well as in the drying stage which was dominated by yeast isolates. The drying stage is also dominated by proteolytic microbes because the itegumen (silver skin) of coffee beans contains polysaccharides (cellulose and hemicellulose), monosaccharides, proteins and polyphenols. Protein hydrolysis by proteolytic microbes produces several types of amino acids which contribute to the formation of flavors in coffee (Pereira et al, 2018).

Table 2. Enzymatic bacterial isolates

No.	Kode Isolat	Pektinase	Selulase	Amilase	Protease
1	B1	++	++++	-	-
2	B2	-	-	+	+
3	B3	+	++	+	-
4	B5	-	-	-	-
5	B7	-	-	-	-
6	B8	+	+	+	-
7	B9	-	-	+	-
8	B10	+	++	++	-
9	B11	-	-	-	+
10	B12	-	-	-	+
11	B13	-	-	-	-
12	B14	-	-	+	+
13	B15	-	-	-	-
14	B18	-	-	-	-

Note: + = there is a clear zone (the more + signs, the bigger the clear zone) - = no clear zone

Based on the results of species dominance analysis and enzymatic tests, 2 dominant isolates were selected, namely bacteria B10 and yeast Y2 because these two isolates had the highest dominance index values with enzyme activity owned by bacteria B10 namely pectinase, cellulase and amylase (Table 2), as well as yeast Y2 isolate which have overall enzyme activity namely pectinase, amylase, cellulase and protease (Table 3). This shows that these two isolates can be used as starter cultures for use in the controlled Robusta coffee fermentation process.

CONCLUTION

In Robusta coffee processing with wet processing (wet) there is a yeast community with a higher percentage (51.7%) than the bacterial community (48.3%). Of the 14 bacterial isolates that were successfully isolated, 71.4% were gram-positive bacteria (10 isolates) and 28.6% were gram-negative bacteria (4 isolates). The microbial community in the wet processing of Robusta coffee has a species diversity index (H') = 2.48, evenness index (E) = 0.74 and dominance index (D) = 0.17. The results of species dominance analysis and enzymatic tests showed that bacterial isolates B10 and yeast Y2 were selected as dominant isolates and could be used as starter cultures in a controlled fermentation process.

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