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Indigenous lactic acid bacteria as a biological control agent to prevent fungi contamination in the fermentation of cocoa beans

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Abstract

The contamination of mycotoxin-producing fungi during cocoa fermentation is a major concern of cocoa bean quality. This research aimed to investigate the effect of indigenous lactic acid bacteria starter culture, *Lactiplantibacillus plantarum* subsp. *plantarum* HL-15, on the fungi population and the quality of cocoa beans. Fermentation of cocoa beans was carried out by four treatments, namely, fermentation without starter culture in the used box, fermentation with starter culture in the used box, fermentation without starter culture in the new box, and fermentation with starter culture in the new box. The population dynamics of yeast, lactic acid bacteria, acetic acid bacteria, and fungi during fermentation were evaluated. The addition of *L. plantarum* subsp. *plantarum* HL-15 ensured the rise of temperature in the fermentation. There were changes in the growth pattern of these microorganisms during fermentation as the effect of starter culture addition. The addition of *L. plantarum* subsp. *plantarum* HL-15 effectively inhibited fungi growth. Fermentation using starter culture in newly made boxes was the most effective method to inhibit fungi growth and improve cocoa bean quality. Fermented cocoa beans produced by this method can adhere to the requirements of first-grade quality cocoa beans based on the Indonesian National Standard.

Keywords: controlled fermentation; mycotoxin-producing antifungal; *Lactiplantibacillus plantarum* subsp. *plantarum* HL-15; microorganisms growth patterns; cocoa bean quality.

Practical Application: *Lactiplantibacillus plantarum* subsp. *plantarum* HL-15 in fermented cocoa beans can suppress fungal growth.

1 INTRODUCTION

Fermentation is important to improve the quality of cocoa beans. Successful fermentation is determined by various factors. During fermentation, cocoa beans are subjected to biochemical changes mainly due to enzymatic reactions. These are mostly contributed by the activity of microorganisms (Akinfala et al., 2020). There are three essential naturally occurring microorganisms, namely, yeast, lactic acid bacteria (LAB), and acetic acid bacteria (AAB). These microorganisms contribute to the formation of flavor and flavor precursors, determining the quality of cocoa beans (Hamdouche et al., 2019; Ho et al., 2018; Miguel et al., 2017).

There is a rising concern regarding the quality and food safety aspects of cocoa and cocoa-related products. One of the aspects with a significant impact is the occurrence of fungi. In improper post-harvest conditions, such as uncontrolled fermentation and interrupted drying, mold can grow and negatively affect the quality and safety of cocoa beans (Akinfala et al., 2020; Ruggirello et al., 2019; Taniwaki et al., 2018). The occurrence of fungi, including *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Trichoderma* sp., *Paecilomyces, Talaromyces, Pseudophitomyces,* and *Cimplicillium* during fermentation, drying, and storage of cocoa beans, has been previously reported (Akinfala et al., 2020; Marwati, 2017; Ruggirello et al., 2019; Taniwaki et al., 2018). These fungi are responsible for the formation of off-flavors and mycotoxins. Mycotoxins have been reported to be carcinogenic, mutagenic, immunotoxic, and teratogenic (Batish et al., 1997). They cause various detrimental effects on human health. An effort to prevent the production of mycotoxin-producing fungi is critical to ensure the quality and safety of cocoa and cocoa-related products for consumption purposes.

The presence of LAB in the fermentation system could inhibit the growth of mycotoxin-producing fungi (Andrés-Barrao et al., 2017; Lefeber et al., 2011; Miguel et al., 2017). Enrichment of LAB in the fermentation system increases lactic acid production (Copetti, 2019). Together with protein compounds from *Saccharomyces cerevisiae* and *Candida ethanolica,* this inhibits the growth of fungi and changes the diversity of

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microbial communities (Copetti, 2019). Based on these advantages, LAB is often used as a starter in processing fermented products (Copetti, 2019). In the fermentation of cocoa beans, adding LAB as a starter could inhibit fungi growth, reduce mycotoxins, and affect the sensory quality of chocolate products (Miguel et al., 2017; Perczak et al., 2018). LAB can improve the performance of fermentation, resulting in a shorter fermentation time. Furthermore, it could improve the quality of cocoa beans suitable for chocolate production (Marwati, 2017). However, further studies are needed in its application on different fermentation systems. Currently, information on the effect of LAB addition on the dynamic population of microorganisms during fermentation and drying and on the quality of cocoa beans is limited.

Among several LAB used as starter cultures, *Lactiplantibacillus plantarum* subsp. *plantarum* and *L. fermentum* are the most utilized species (García-Ríos et al., 2021; Hamdouche et al., 2019). The strain of *L. plantarum* ITEM 17215 was superior in inhibiting the growth of *Penicillium roqueforti* and *Mucor circinelloides* (Quattrini et al., 2018). A previous study showed that fungi growth and contamination of ochratoxin in cheddar cheese, vegetables, and fresh meat could be controlled by the use of *L. plantarum* CRL 778 (Dallagnol et al., 2019). *Lactiplantibacillus plantarum* subsp. *plantarum* HL-15 is a LAB naturally occurring in cocoa fermentation. This strain was isolated from fermented cocoa beans in the Farmer group of Hargo Mulyo I, Patuk Sub-district, Gunungkidul Regency, Yogyakarta Province, Indonesia. This isolate can inhibit the growth of *Aspergillus niger*, which is one of the most prevalent mycotoxin producers. The potential of this strain as a starter culture for cocoa fermentation has been studied (Dallagnol et al., 2019). The addition of *L. plantarum* HL-15 starter culture, either singly or simultaneously with the addition of *Candida famata* HY-37 and *Acetobacter* spp. HA-37, could suppress the growth of *A. niger* YAC-9 and prevent the synthesis of ochratoxin A in small-scale cocoa fermentation (Jamili et al., 2016).

In this study, the use of *L. plantarum* HL-15 to improve the quality of fermented cocoa beans and as a mycotoxin control agent in farmer-scale processing (40 kg capacity) was studied. New and used fermentation boxes were used to evaluate the effect of *L. plantarum* HL-15 addition in new and existing microorganism communities. The effect of *L. plantarum* HL-15 on the population dynamic of yeast, LAB, AAB, and fungal population and cocoa bean quality is discussed in order to better understand its role in cocoa fermentation. The result of this study is important to evaluate the feasibility of using *L. plantarum* HL-15 in a real-life application for its usability in farmer and industrial-level processing.

2 MATERIALS AND METHODS

2.1 Cocoa beans

Fully ripe Forastero cocoa pods were obtained from smallholder plantations in Gunungkidul Regency, Yogyakarta, Indonesia. Wet cocoa bean for fermentation was obtained using procedures according to Good Handling Practices (Djaafar et al., 2019).

2.2 Growth media

The media used for microbiological analysis were based on the study by Jamili et al. (2016). These included de Man Rogosa Sharpe Agar (MRSA) (MERCK Darmstadt, Germany), de Man Rogosa Sharpe broth (MRSB) (MERCK Darmstadt, Germany), Peptone Glucose Yeast Extract Agar (PGYA) (Basing-stoke, England), Malt Extract Agar (MERCK Darmstadt, Germany), and Dichloran Rose Bengal Chloramphenicol Agar (MERCK Darmstadt, Germany).

2.3 Preparation of indigenous Lactiplantibacillus plantarum subsp. plantarum HL-15 for cocoa bean fermentation

Lactiplantibacillus plantarum subsp. *plantarum* HL-15 used in this study was an original culture isolated from cocoa bean fermentation. This was stored at the Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta, Indonesia. The isolation of the culture was reported in the previous study (Dallagnol et al., 2019). Culture rejuvenation was initiated by taking 0.1 mL of the culture in a cryotube. It was then inoculated in 1 mL of sterile MRS broth and incubated at 37°C for 24 h. Subsequently, rejuvenation was carried out in stages until 10% of the culture was transferred to an Erlenmeyer flask containing 500 mL of growth medium. This culture was incubated at 37°C for 24 h. The concentration of starter culture used for cocoa bean fermentation was 10^9 CFU/mL (Andrews, 1992; Marwati et al., 2019; Rahayu et al., 2021).

2.4 Fermentation of cocoa beans

A total of 40 kg of wet cocoa beans were used for fermentation. The fermentation was done based on the previous study by Hatmi et al. (2015) and Marwati et al. (2020, 2021). Newly made wooden boxes were used alongside the ones currently used for cocoa fermentation. The cocoa beans were put into boxes, with or without adding *L. plantarum* subsp. *plantarum* HL-15, according to the treatment. Four treatments in cocoa bean fermentation were carried out, including fermentation without *L. plantarum* subsp. *plantarum* HL-15 in the used box (K1), fermentation with *L. plantarum* subsp. *plantarum* HL-15 in the used box (K2), fermentation without *L. plantarum* subsp. *plantarum* HL-15 in the new box (K3), and fermentation with *L. plantarum* subsp. *plantarum* HL-15 in the new box (K4). In the treatment involving *L. plantarum* subsp. *plantarum* HL-15, as much as 500 mL of the starter culture was added. On the contrary, 500 mL of water was used in the treatment without a starter culture. The experimental design used was a completely randomized design with three replications. The data are expressed as the average value of three replications with standard deviation.

2.5 Drying of fermented cocoa beans

Fermented cocoa beans were placed on woven bamboo (120 × 70 cm) on a raised bed dryer. The beans were evenly spread in one layer and sun-dried. When drying, cocoa beans were turned 1–2 times a day. The drying was conducted for 4 days (8–9 h/day of effective sunshine) to reach the moisture content of 7.5%.

2.6 Temperature and pH measurement

The temperature and pH were measured every 24 h during 5 days of fermentation. The measurements were carried out at the top, middle, and bottom of the cocoa beans pile in fermentation boxes using a thermometer and pH meter (ATC, Jakarta) (Marwati et al., 2021; Purwanto et al., 2019).

2.7 Microbiological analysis

The microbiological analyses were conducted for yeast, LAB, AAB, and fungi populations. The analysis was carried out on the samples obtained every 24 h during the fermentation and drying of the cocoa beans. The process of microbiological analysis included sample refining by mixing 40 g of cocoa beans with 60 mL of sterile NaCl (0.85%). It was then added with 300 mL of sterile NaCl (0.85%) and stirred until homogeneous. The sample solution was then diluted with sterile NaCl (0.85%) to a dilution level of 10^{-9}. Each dilution was plated to a Petri dish using the pour plate method (Eijlander et al., 2020; Jamili et al., 2016; Marwati et al., 2019). Incubation was carried out at 37°C for 48 h. For fungi growth analysis, incubation was carried out for 5 days at room temperature. Colony enumerations were counted using the Quebec Colony Counter.

2.8 Quality determination of cocoa beans

Quality determination of cocoa beans was done using 100 g of cocoa beans. Visual measurements of cocoa beans were carried out on the parameters of slaty, moldy, germinated, and insect-containing beans. The parameters of analysis followed the Indonesian National Standard 2323:2008/Amd I:2010 (Marwati et al., 2019, 2020).

3 RESULTS

3.1 Temperature and pH during fermentation

Fermentation using the used box and without the addition of starter culture (K1) had different temperature and pH profiles compared with the other treatments (Figure 1A). The fermentation of cocoa beans in K1 was less intensive than others. This was indicated by a prolonged increase in temperature and a decrease in pH. Fermentation of cocoa beans using the starter culture in the new box (K4) reached a temperature above 40°C in 3 days and persisted until the end of fermentation. In the fermentation using a new box without a starter culture (K3) and a used box with a starter culture (K2), the highest temperature was reached on the third and fourth days, respectively. However, the temperatures then decreased until the fifth day. These conditions were different from that of K1.

The pH values of cocoa pulp were in the range of 3.15–3.40 at the start of fermentation and decreased to the range of 2.90–3.45 at the end of fermentation (Figure 1B). The initial pH of cocoa pulp in the fermentation using used and new boxes without starter culture was similar. On the contrary, the pH of cocoa pulp in the fermentation using *L. plantarum* subsp. *plantarum* HL-15 was slightly higher than that without the addition of culture. However, the pH value of cocoa pulp in the fermentation using starter culture was lower than that without starter culture at the end of fermentation.

3.2 Population dynamic of microorganisms during fermentation

There was a similarity in the pattern of the population dynamic of yeast, AAB, and fungi in cocoa bean fermentation from different treatments (Figures 2A–2C). The LAB population showed different patterns due to the addition of starter culture (Figure 2B). Fermentation using new boxes showed less fungi population than used boxes. The yeast population peaked (7.29–8.60 log CFU/g) at the first 24 h of fermentation (Figure 2A) and then decreased after 24 h until it was undetectable at the end of fermentation (K2 and K4).

Fermentations without *L. plantarum* HL-15 (K1 and K3) had the same LAB population on the first day (3 log CFU/g). In those treatments, the LAB population increased until it peaked at day 3. The numbers were then decreased until the end of fermentation. Meanwhile, a high LAB population was evaluated

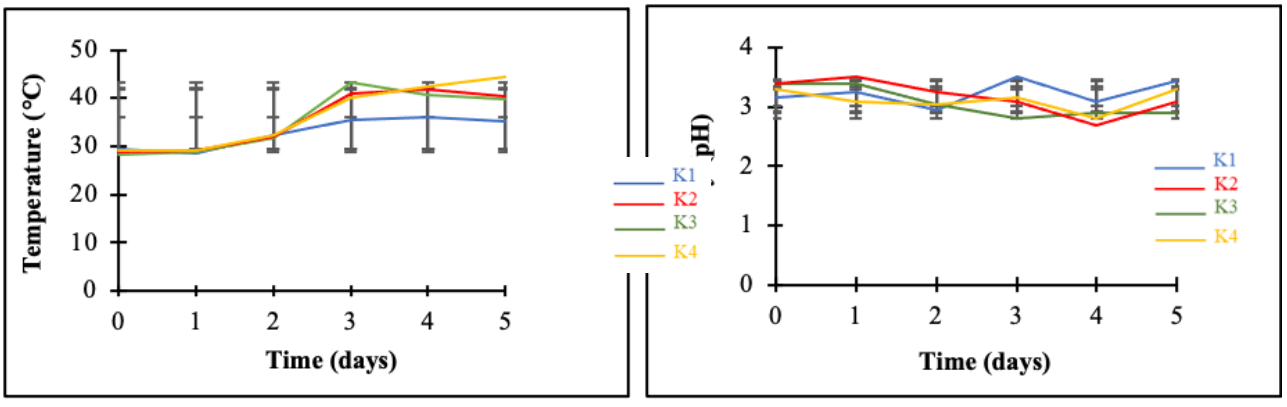

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Figure 1. (A) The temperature and (B) the pH of a pile of cocoa beans in fermentation without *Lactiplantibacillus plantarum* subsp. *plantarum* HL-15 in the used box (K1); fermentation with *L. plantarum* subsp. *plantarum* HL-15 in the used box (K2); fermentation without *L. plantarum* subsp. *plantarum* HL-15 in the new box (K3); and fermentation with *L. plantarum* subsp. *plantarum* HL-15 in the new box (K4).

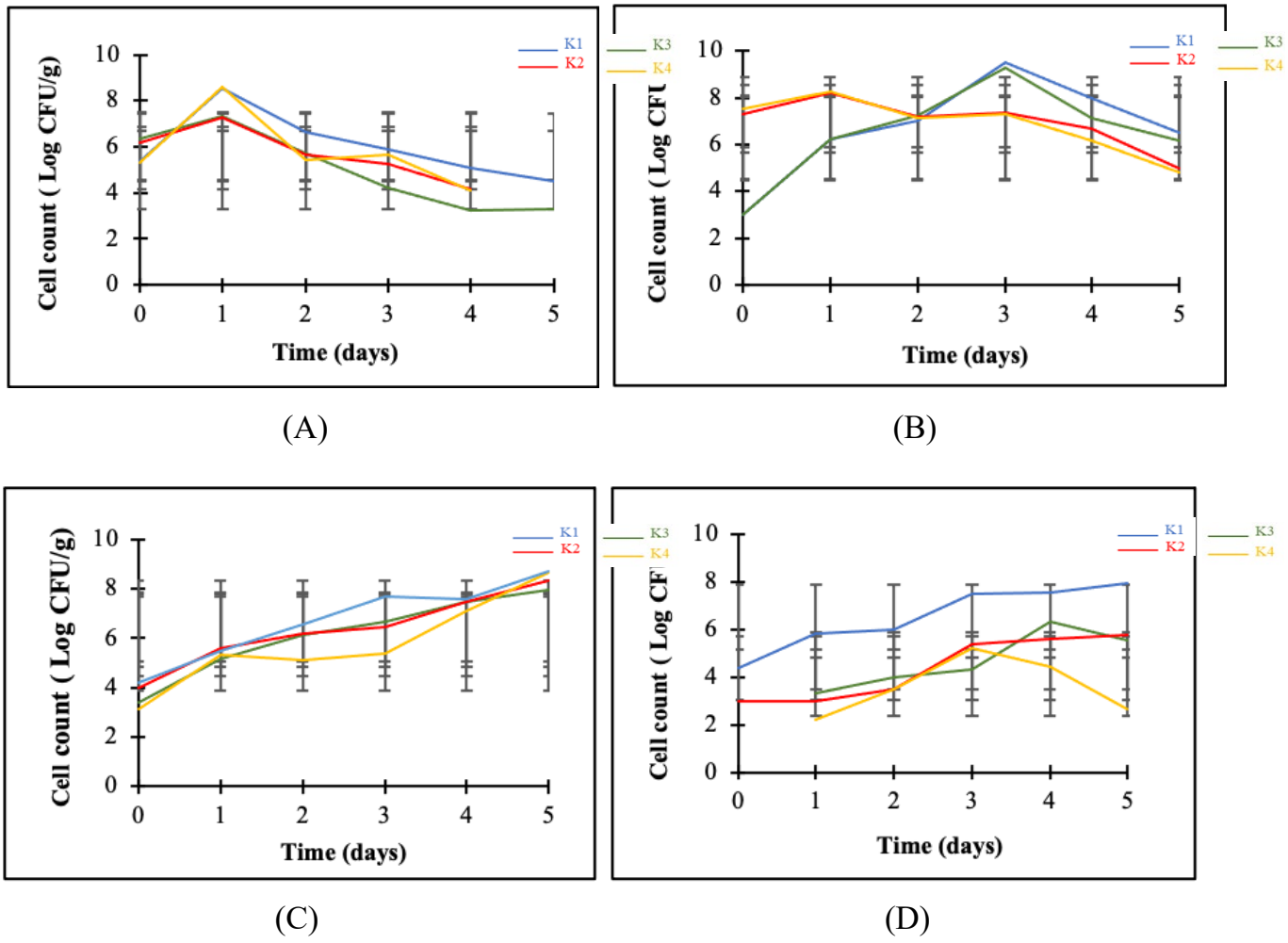

Figure 2. The growth patterns of (A) yeast, (B) lactic acid bacteria, (C) acetic acid bacteria, and (D) fungi during cocoa bean fermentation without *Lactiplantibacillus plantarum* subsp. *plantarum* HL-15 in the used box (K1); fermentation with *L. plantarum* subsp. *plantarum* HL-15 in the used box (K2); fermentation without *L. plantarum* subsp. *plantarum* HL-15 in the new box (K3); and fermentation with *L. plantarum* subsp. *plantarum* HL-15 in the new box (K4).

in treatments K2 and K4 due to the addition of starter culture. However, the population then decreased. The LAB population declined greatly after 3 days of fermentation, such as K1 and K3. At the end of fermentation, K2 and K4 had a lower LAB population (5 and 4.80 log CFU/g) than K1 and K3.

The AAB population increased during fermentation in all treatments (Figure 2C). The population of AAB in the fermentation with a starter culture (K2 and K4) was relatively constant on 48–72 h of fermentation before increasing until the end of fermentation. The fungi population in K1 and K2 treatment was detected at the beginning and continued to increase until the end of fermentation. Meanwhile, no fungi were detected at the start of fermentation in the new boxes (K3 and K4). Fungi were detected after 24 h of fermentation in treatments K3 and K4. Fungi growth was lower in the fermentation using *L. plantarum* HL-15 (K2 and K4) than those without a starter (K1 and K3). The lowest fungi population at the end of fermentation was evaluated in the treatment combining a starter culture and a new fermentation box (K4) (Figure 2D).

3.3 Population dynamic of microorganisms during drying

There was a variation in the growth patterns of yeast, LAB, AAB, and fungi during drying in different treatments (Figure 3). Yeast populations began to be detected after 24 h of drying in cocoa beans fermented in the new boxes. This condition is different for fermentation using used boxes (K1 and K2) that showed the existence of yeast at the start of drying. Cocoa beans fermented with and without LAB showed similar population patterns until 48 h of drying. They were in the range of 4.81–5.21 log CFU/g. Cocoa beans fermented using used boxes (K1 and K2) had a higher LAB population (4.78 and 4.34 log CFU/g, respectively) than that of using new boxes (~3 log CFU/g) at the end of fermentation.

The AAB populations in cocoa beans were relatively stable during drying in all treatments. This was like that of LAB populations. AAB population increased in all treatments in the first 24 h, except until 48 h for K1. K2 and K4 had the lowest population (4.53 and 4.49 log CFU/g, respectively), and K1 treatment had the highest (5.15 log CFU/g) at the end of drying.

The trend of fungi population in cocoa beans from all treatments during drying was similar, except for the K3 treatment. Cocoa beans from the K3 did not have any fungi until the second day of drying. However, its population increased dramatically afterward. The highest fungi population at the end of the drying was evaluated in cocoa beans from K1. From lowest to highest, the fungi population of cocoa beans were 2.70, 2.85, 3.00, and 3.20 log CFU/g for K4, K3, K2, and K1, respectively.

3.4 Quality of cocoa beans

There were variations in the quality of cocoa beans from all fermentation treatments (Table 1). According to the Indonesian standard for cocoa beans, the maximum limit of moldy, slaty, germinating, and insect-containing beans of first-grade quality is 2%, 3%, 2%, and 1%, respectively. Among the treatments, cocoa beans from K2, K3, and K4 were of first-grade quality. On the contrary, a high proportion of slaty beans was found

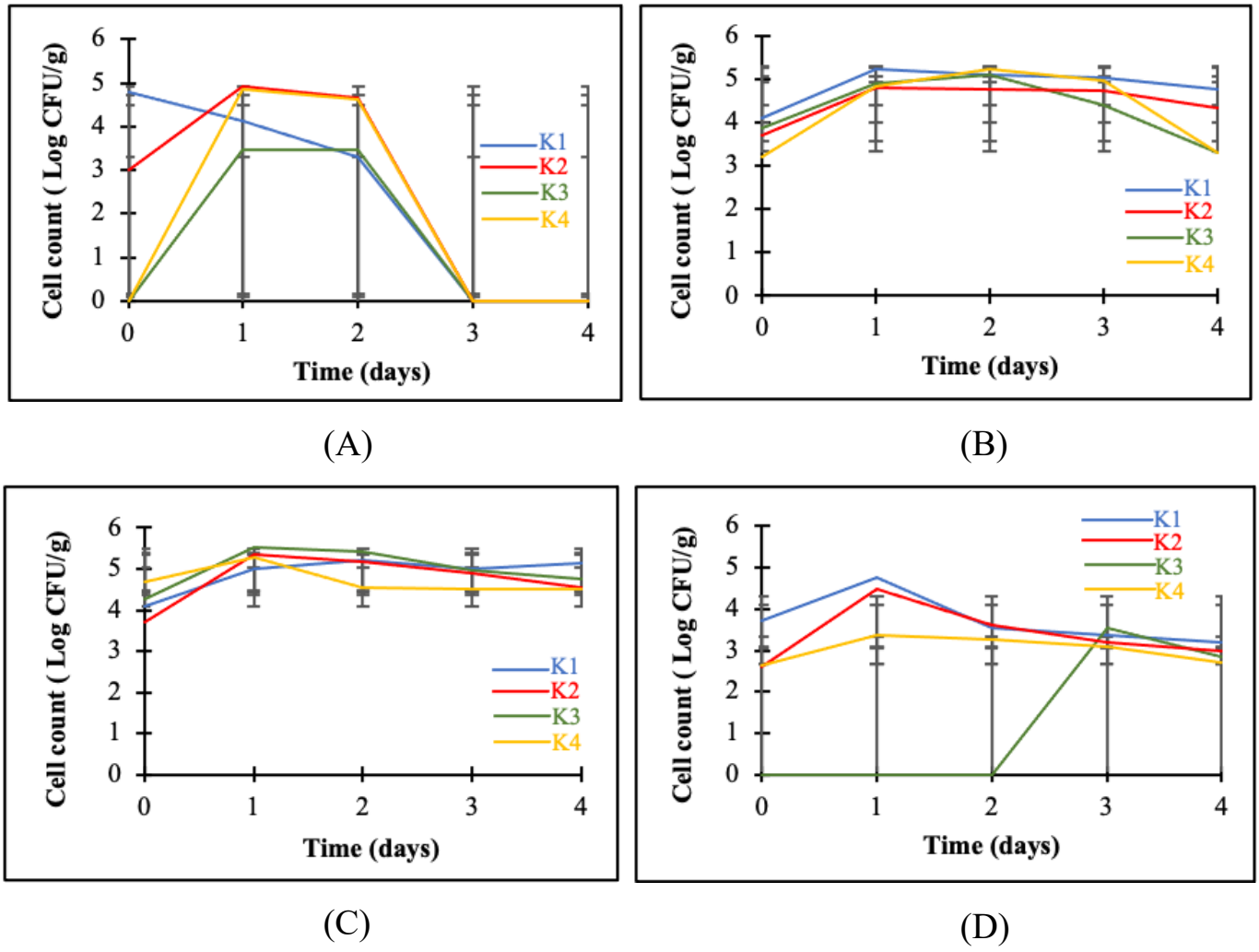

Figure 3. The growth patterns of (A) yeast, (B) LAB, (C) AAB, and (D) fungi during drying after cocoa bean fermentation without *Lactiplantibacillus plantarum* subsp. *plantarum* HL-15 in the used box (K1); fermentation with *L. plantarum* subsp. *plantarum* HL-15 in the used box (K2); fermentation without *L. plantarum* subsp. *plantarum* HL-15 in the new box (K3); and fermentation with *L. plantarum* subsp. *plantarum* HL-15 in the new box (K4).

Table 1. The quality of fermented cocoa beans.

Fermentation	Analysis results					
	Number of beans/100 g	Moldy bean (%)	Slaty bean (%)	Germinated bean (%)	Insect-containing bean (%)	Broken bean (%)
K1	105 ± 2.12	0	4.29	0	0	0
K2	110 ± 0.71	0	0.92	0	0	0
K3	107 ± 7.78	0	1.39	0	0	0
K4	**99 ± 5.66**	**0**	**1.01**	**0**	**0**	**0**

K1: fermentation without *Lactiplantibacillus plantarum* subsp. *plantarum* HL-15 in the used box; K2: fermentation with *L. plantarum* subsp. *plantarum* HL-15 in the used box; K3: fermentation without *L. plantarum* subsp. *plantarum* HL-15 in the new box; K4: fermentation with *L. plantarum* subsp. *plantarum* HL-15 in the new box.

in the treatment without *L. plantarum* HL-15 and using used box (K1).

4 DISCUSSION

The addition of starter culture *L. plantarum* subsp. *plantarum* HL-15 accelerated the increase in the temperature of fermentation. It resulted in a higher final temperature than the one without a starter culture. Fermentation using the new box and *L. plantarum* HL-15 could maintain the high temperatures (> 40°C) from 3 days until the end of fermentation. Temperature is an essential factor for successful fermentation. The increase in temperature indicated that exothermic reactions take place during fermentation. This ensures the growth of important microorganisms during fermentation, resulting in the formation of important chemicals to improve the quality of cocoa beans (Ouattara et al., 2019).

Temperature and incubation time influenced the modulation of LAB growth to produce antifungal agents such as organic acids (Batish et al., 1997). This was supported by the decreasing trend in pH during fermentation. The pH value of cocoa pulp at the beginning of fermentation was 3.15–3.40. On the fifth day of fermentation, the pH values were 2.90–3.45 (Figure 2). The pH of cocoa pulp at the start of fermentation was similar in all treatments (Figure 2). K1 treatment showed an increase in pH during fermentation. The increment in pH of cocoa pulp was caused by ethanol production, secretion of pectinolytic enzymes, and citric acid metabolism by yeasts (Copetti, 2019). However, the addition of LAB led to a sharp decrease in pH. This was due to the formation of organic acids by LAB (Rahayu et al., 2021).

In this study, the succession of microorganisms during fermentation without *L. plantarum* HL-15 followed the trend of spontaneous fermentation previously reported (Andrés-Barrao et al., 2017; Ren et al., 2020). Yeast dominated during the first 48 h of fermentation, followed by LAB (48–96 h) and AAB (> 96 h). This was in agreement with the result of a previous study (Miguel et al., 2017; Pereira et al., 2017; Rhee et al., 2011). Yeast is useful as a bioprocessing agent in food processing, especially fermentation (Copetti, 2019). They are involved in ethanolic metabolisms. Together with acid and heat, ethanol induces bean death and triggers enzymatic degradation in cocoa beans (Andrés-Barrao et al., 2017; Nodem Sohanang et al., 2021). After 48 h, yeast growth was inhibited due to increased alcohol content and aeration (Schwan & Wheals, 2004). The absence of yeast caused the increase in the LAB population to reach its peak in 72 h. This was in agreement with a previous study (García-Ríos et al., 2021). However, this was slower than the results of Schwan and Wheals (2004), who stated that the LAB population peaked in 16–48 h after fermentation. The differences may be attributed to different geographical conditions where the fermentation was carried out. On the contrary, AAB grew steadily during fermentation and reached its peak on the last day of fermentation. Acetic acid bacteria in the fermentation of cocoa beans were dominated by *Acetobacter* species (Djaafar et al., 2019). Acetic acid bacteria is the second major microorganism after LAB in the fermentation of cocoa beans. Its growth in fermentation is independent of the availability of LAB. Acetic acid is the main product of AAB metabolisms, mainly from the oxidation of ethanol produced by yeast (Ho et al., 2018; Schwan & Wheals, 2004). Acetic acid diffuses into the beans, contributing to the overall sensory properties of cocoa beans (Purwanto et al., 2019). The notable aspect of this spontaneous fermentation was the gradual increase in the fungi population. In K1 treatment, the fungi population increased over fermentation time and was a dominant microorganism alongside AAB at the end of fermentation. This increased the risk of dominant fungi metabolisms, including mycotoxin formation in the cocoa beans.

The addition of starter culture *L. plantarum* HL-15 significantly changed the microbial population in the cocoa bean fermentation. LAB and yeast were dominant in the first 48 h of fermentation (Rhee et al., 2011). LAB remained dominant until 72 h. Its dominance also affected the decrease in yeast population after 24 h of fermentation. Lactic acid bacteria were reported to induce stunted yeast growth (Ho et al., 2018). He et al. (Andrews, 1992) found that the addition of LAB inoculums negatively affected the growth of several yeast species. *Hanseniaspora guilliermondi* was detected until the fourth day of spontaneous fermentation but absent after 3 days of fermentation with the addition of LAB. Other yeast species, such as *Kluyveromyces marxianus* and *Phicia kudriavzevii*, were not affected by the addition of inoculants and remained until the sixth day of fermentation (Andrews, 1992). A decrease in yeast population may be affected by the formation of lactic acid (decrease of pH) and the increase in temperature (Hamdouche et al., 2019; Pereira et al., 2017). *Kloeckera apis* grew only at the early stages of fermentation (first 24–36 h) due to its low resistance to high temperatures. On the contrary, *S. cerevisiae* and *Candida tropicalis* were more heat resistant and remained active at the temperature of 40–47°C. However, the absence of yeast in K2 and K4 treatments in this study might result from quorum sensing of LAB toward yeasts. Acetic acid bacteria growth was also suppressed during LAB dominance (0–72 h) and reached its population peak only after 96 h of fermentation. Surprisingly, the combination of LAB addition and the use of a new box could suppress the fungi population to only ~2 Log CFU/g at the end of fermentation.

The use of used and newly made boxes affected the initial microbial population in the fermentation of cocoa beans, especially fungi. Used boxes might have fungi and fungi spores utilizing wooden boxes as an immobile matrix. Those fungi and spores were then activated by the moisture from wet cocoa beans, resulting in a spike in the fungi population at the start of fermentation. Fungi growth during the initial stage of fermentation was reported to be limited by the temperature and ethanol concentration (Pereira et al., 2017). This initial fungi population is important as it may affect the yeast, LAB, and AAB's quorum sensing. It prevented their antifungal activity, resulting in a persistent population of fungi until the end of fermentation. Indeed, the fermentation boxes should be cleaned periodically to prevent the buildup of the fungi population.

During the drying process, yeast was undetectable after 3 days of drying. The drying process causes a decrease in the moisture content of cocoa beans, suppressing the growth of yeast. The populations of AAB and LAB were relatively stable

during drying. This was in agreement with the study by Hamdouche et al. (2019), who found that LAB such as *L. plantarum*, *K. cryocrescens*, and *Weissella* sp. can still be detected at 2–4 days of drying. At the same time, AABs that can be detected during drying were *Gluconobacter xylinum* and *Clostridium* sp. (Akinfala et al., 2020). However, the population of each of these bacteria dynamically changed until the last day of drying. Fungi were still present after the drying process. This was in line with the study by Delgado-Ospina et al. (2020). Fungi can survive under aerobic conditions and low water activity (Aw). However, the fungi population in the cocoa beans after drying was relatively similar for all treatments.

The similarity in the final population of fungi after drying should not be associated with the ineffectiveness of the addition of LAB to prevent the fungi growth. The antifungal activity of LAB works with various mechanisms, such as quorum sensing and the production of antifungal agents. LAB and AAB produce extracellular organic acids during fermentation that can suppress the growth of fungi (Rhee et al., 2011). These organic acids from *L. plantarum* HL-15 were reported to suppress the growth of *A. niger,* an ochratoxin A producer (Rahayu et al., 2021). On the contrary, acetic acid produced during fermentation could suppress the growth of fungi of *Aspergillus carbonarius* and *A. niger* species (Taniwaki et al., 2018). Hence, the occurrence of these acids may provide protection against fungi buildup during cocoa bean storage. As the diversity of microorganisms was also not evaluated in this study, the fungi that remained after drying may not contribute to mycotoxin production. However, further studies on the detailed evaluation of microorganisms' diversity and their secondary metabolites are needed to confirm this hypothesis.

In terms of quality, the cocoa beans produced for all treatments were free from moldy, germinated, insect-containing, and broken beans. It conformed to the requirement of Indonesian National Standard (SNI) 2323: 2008/Amd1: 2010. This was similar to the previous studies (Marwati et al., 2020, 2021; Rahayu et al., 2021). All the treatments successfully induced bean death in cocoa beans, resulting in non-germinated beans. Furthermore, all the treatments produced a low proportion of unfermented beans (slaty). Fermentation without *L. plantarum* HL-15 on the used box produced a slightly higher proportion of slaty beans. This indicated that the addition of *L. plantarum* HL-15 effectively improved the fermentation performance, resulting in a better physical quality of cocoa beans.

5 CONCLUSION

This study reported that adding *L. plantarum* subsp. *plantarum* HL-15 as a starter culture could modify the population dynamics of microorganisms during fermentation. It could inhibit fungi growth. The use of used boxes without prior cleaning was not recommended due to the buildup of the fungi population in the wooden matrix. A combination of the use of starter culture and newly made boxes produced high-quality cocoa beans. Therefore, adding *L. plantarum* subsp. *plantarum* HL-15 as an indigenous LAB starter culture could be applied to improve the fermentation of cocoa beans. However, further studies are needed to better understand the antifungal mechanisms in cocoa beans resulting from LAB addition. The results of this study are important to improve the quality of fermented cocoa beans for exports and chocolate-manufacturer uses.

6 PATENTS

a. Patent: Dry Starter Manufacturing Process of *L. plantarum* HL-15 for Mycotoxin Producing Fungus Control, 2017 (IDS000001851) (in Indonesian)

b. Patent Cocoa Bean Fermentation Process with The Addition of Starter *L. plantarum* HL-15 to Inhibit Fungi Growth, 2019 (IDS000002554) (in Indonesian)

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