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Increase in Polyphenolic Substances from Fermented Robusta Coffee Pulp (*Coffea canephora* L.) by Using Indigenous Actinomycetes

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ABSTRACT

The fermentation of the coffee fruit processing into coffee beans leaves the fermented coffee pulp which still contains polyphenol secondary metabolites. This study aimed to analyze the total flavonoid content (TFC) and total phenolic content (TPC) of fermented robusta coffee pulp (*Coffea canephora* L.) by using indigenous Actinomycetes. The ability of fermented extracts to inhibit free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) also were done. In this study, coffee cherries were fermented in the solid-state fermentation cultivation (SSF) using isolates HJ4.5b, P2b(b).3 and P2b(b).18 which in previous studies were reported to have cellulolytic and xylanolytic activities. Determination of molecular identification base on 16S rRNA gen showed the isolates HJ4.5b and P2b(b).3 have similarities to the genus *Streptomycetes*, while P2b(b).18 has a homologous base arrangement with a rare actinomycetes genus Micromonospora. Fermentation using P2b(b).3 on the 9th day of fermentation indicated the highest percentage increase in TFC (295.54%) with IC_{50} 18.41 µl/ml and having an antioxidant activity index (AAI) value of 2.14 which was included in the very strong antioxidant activity category.

1. Introduction

Indonesia is the fourth largest producer and exporter of coffee after Brazil, Vietnam, and Colombia (Jamil 2019). Recently, coffee is a drink that has attracted attention because of its positive effects on health that have been widely studied, resulting in an increase in the culture of drinking coffee around the world. This is followed by an estimated increase in global coffee production from 9.5 million tonnes to 10.2 million tonnes in 2018-2019 (Adam *et al.* 2020). This global phenomenon also has an effect on increased waste generated during the coffee preparation process. Coffee by-product which is still rich in organic components can be toxic and cause serious environmental and health problems if not handled properly (Woldesenbet *et al.* 2016).

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Improving the quality of coffee pulp as a potential polyphenol producer needs to be done in an effort to get better pharmacological activity. Fermentation with various types of microbes and methods has been reported to affect polyphenol levels and antioxidant activity of coffee pulp extract (Arellano-Gonzales *et al.* 2011; Garcia *et al.* 2015; Lopez *et al.* 2013; Oktaviani *et al.* 2020; Santos *et al.* 2019). However, the fermentation of coffee pulp using the Actinomycetes of *Streptomyces* sp. until now only reported by Kurniawati *et al.* (2016) and Orozco *et al.* (2008). Actinomycetes are filamentous bacteria which in addition to having a high ability to produce antibiotic bioactive compounds, are also capable of producing various hydrolytic enzymes so that they are potential microorganisms that can be used as biological agents in biotechnology processes (Saini *et al.* 2015).

Putri *et al.* (2019) reported three potential Actinomycetes isolates (HJ4.5b, P2b(b).3, and

P2b(b).18) capable of producing cellulase and xylanase using coffee pulp substrate on solid media. The ability of the three selected isolates needs to be further applied to coffee cherries fermentation. The purpose of this study was to ferment the coffee cherries using the three potential selected isolates using SSF method. The fermentation performance in this research report was limited to the analysis of TFC and TPC. DPPH free radical inhibition activity from fermented extracts and molecular identification of the three isolates were also carried out.

2. Materials and Methods

2.1. Materials

The isolates used in this study were HJ4.5b, P2b(b).3, and P2b(b).18 which based on previous studies showed cellulase and xylanase activity. Coffee cherries sampled from smallholder plantations in the city of Pagaralam, South Sumatra in February 2020.

2.2. Identification Molecular of Selected Isolates

Genomic DNA isolation of isolates was carried out according to manufacturer's protocol using Presto Mini gDNA and Zymo Research (ZR) gDNA bacteria kit. Nanodrop 2,000 spectrophotometer (Thermo Scientific, USA) was used to evaluate the concentration and purity of genomic DNA. The genomic DNA that had been isolated was amplified using the Polymerase Chain Reaction (PCR) method. The 16S- spesific primers for actinobacteria were used for PCR amplification. Primer 27F (AGAGTTTGATCCTGGCTCAG) (Bruce *et al.* 1992), primer 16Sact1114R (GAGTTGACCCCGGCRGT) (Martina *et al.* 2008), primer 1492R (TACGGTTACCTTGTTACGACTT) (Payal *et al.* 2014).

Two different of amplification PCR Gotag Green Master Mix and Mytag Red Mix (Bioline) (Janatiningrum *et al.* 2018) were used in this study. The PCR products were analyzed on 1% agarose gel electrophoresis and used Ethidium Bromida (EtBr) for staining. The visualization of the amplification results was carried out using a UV transilluminator. The PCR products were sent to the sequencing company (PT. Genetika Science Indonesia) to determine the sequens of RNA segment of selected Actinomycetes. The sequencing results were entered into the Basic Local Alignment Search Tool (BLAST) program from National Center for Biotechnology Information (NCBI) to compare the nucleotide sequences to sequence databases and calculates the statistical significance. Molecular Evalutionary Genetic Analysis (MEGA) version 7.0 software was used for construction the phylogenetic tree with a 1,000 replication-bootstrap of the neighbor-joining method (Saitou and Nei 1987).

2.3. Fermentation of Coffee Cherries Using Selected Actinomycetes

Three selected isolates were rejuvenated on the ISP4 agar medium. The media that had been scratched with isolates were then incubated for 4 days. A total of 1 corckborer (0.5 cm diameter) of selected isolates, each inoculated into 50 ml of liquid media (in 100 ml Erlenmeyer) supplemented by 2% powder of dry coffee pulp. The media containing the culture was then incubated for 6 days at 27°C using an incubator shaker (IKA KS4000i, Germany) at a speed of 120 rpm. On the 6th day, starter culture was inoculated in each coffee cherries fermentation treatment group.

Furthermore, for cultivation, 500 g of freshly harvested red ripe coffee cherries were used. The selected coffee cherries were then washed under running water, and drained until the remaining water was dry. Then weighed for each treatment in the amount of 500 g. The coffee cherries were then sterilized using a UV light for 60 minutes and then put into a fermentation container (Kurniawati *et al.* 2016).

The starter cultures of each isolate aged 6 days were then inoculated in a fermentation container containing 500 g of sterilized coffee. The volume of inoculum introduced was 10% (v/w) of the weight of the substrate. The fermentation process was carried out for 9 days at room temperature and placed in a dark room. Stirring every 24 hours so that all the surface of the coffee cherries was exposed to the liquid inoculum media and the growth of the inoculum was evenly distributed. Evaluation of the performance of the fermentation results was carried out on days 0, 1, 2, 6, and 9. All treatments were carried out a triple.

2.4. Extraction of Fermented Coffee Pulp

The coffee cherries were peeled to separate the coffee pulp from the coffee beans. The coffee pulp was then dried in an oven at 50°C for 48 hours. Complete drying then followed by direct sun drying for 2 days. The coffee pulp was then ground and filtered with

a size of 100 mesh. The dry powder of coffee pulp macerated with 96% ethanol solvent and aquadest (80:20) for 24 hours at room temperature and then filtered. The maceration process was carried out 3 times. The macerate was then dried with a rotary evaporator (Eyela N1000, Japan) so that a thick extract was obtained and followed by drying the extract with a freeze dryer (Eyela FDU1200, Japan). The extract was then stored in a dark bottle for the determination of TFC, TPC, and antioxidant activity.

2.5. Determination of Total Flavonoid Content (TFC) and Total Phenolic Content (TPC)

Measurement of TFC was carried out by modifying the colorimetric method of aluminum chloride (Madaan and Kumar 2011) and rutin was used as a standard compound. Sample solution (1,000 µg/ml in methanol) 0.5 ml was added with 1.5 ml of methanol, 0.1 ml of 10% AlCl3 solution, 0.1 ml of 1M potassium acetate solution and 2.8 ml of distilled water. Then the absorbance was measuring using UV/VIS spectrophotometer (Hitachi U 2910, Japan) at the maximum wavelength of the rutin (416 nm). The TFC was expressed in rutin equivalents (mgRE/g of extract).

The TPC was determined by the Follin-Ciocalteu method with gallic acid as a standard based on Madaan and Kumar (2011), with modifications. About 0.3 ml of each sample solution (1,000 µg/ml) were added with 1.5 mLlFollin-Ciocalteu reagent 1:10. After shaking well and were left to stand for 3 minutes and then added 1.2 ml of 7% Na_2CO_3 solution. The absorbance was measuring at the maximum wavelength of gallic acid (764.5 nm). The TPC expressed in gallic acid equivalents (mgGAE/g extract).

2.6. Antioxidant Activity

The stable DPPH radical was used for the determination of free radical scavenging activity of fermented extract (Vasic *et al.* 2012). The fermented extract solutions were prepared in various concentrations in methanol. Then 2 ml of the test sample were added with 2 ml of DPPH solution in methanol (0.1 mM). Then incubated at room temperature for 30 minutes in a dark room. The absorbance was measured at a wavelength of the stable 0,1 mM DPPH solution in methanol (515.5 nm). The control was 2 ml of DPPH in 2 ml of methanol solution. The percentage value of inhibition was regressed in the linear regression equation to get the value of Inhibitory Concentration (IC_{50}). Inhibition activity (%) = 100% × [(control abs - sample abs)/ control abs)]. Antioxidant activity was determined based on the antioxidant activity index (AAI) (Scherer and Godoy 2009).

2.7. Statistical Analysis

All data were expressed as means ± standard deviations. The differences between the means were analyzed by ANOVA followed by Duncan's analysis ($P \leq 0.05$) using Excel for Windows. Correlation between the antioxidant activity and contents of polyphenol was conducted by Pearson's correlation coefficient using the Excel program for Windows.

3. Results

3.1. Identification of Selected Isolates

The amplification PCR of DNA isolated from HJ4.5b and P2b(b).3 using Gotag green master mix, while P2b(b).18 using Mytag red mix. The visualization of the amplification results of HJ4.5b, P2b(b).3, and P2b(b).18 showed the single band and all the amplified DNA had a length of about 1,500 base pairs. These were in accordance with the target identification of the 16S rRNA gene (Figure 1). The PCR amplification product was then analyzed for its nucleotide sequence by the sequencing method. Sequencing analysis after consensus sequencing of the two primers used resulted in nucleotide lengths ranging from 1,056 to 1,500 base pairs. These results were then compared with the NCBI BLAST GenBank database and the results showed that HJ4.5b and P2b (b).3 belong to the genus *Streptomyces* and P2b (b).18 was the genus *Micromonospora*. The base sequences HJ4.5b and P2b (b).3 had similarities in the composition of the bases respectively with *Streptomyces jiujiangensis* JXJ 0074 (99.04%) and *Streptomyces jeddahensis* G25 (99.04%), while the base sequences P2b(b).18 was confirmed to be similar to the rare actinomycetes belong to *Micromonospora* sp strain SB (99.25%) (Table 1).

Analysis of phylogenetic tree with bootstrap 1000x showed that isolate HJ4.5b and P2b.(b).3 were closely related to *S. jiujiangensis* strain JXJ 0074 and *S. jeddahensis* strain G25 with a value of 100% bootstrap respectively. P2b(b).18 had a value of 100% bootstrap with *Micromonospora* sp. stran SB. Based on phylogenetic tree analysis (Figure 2).

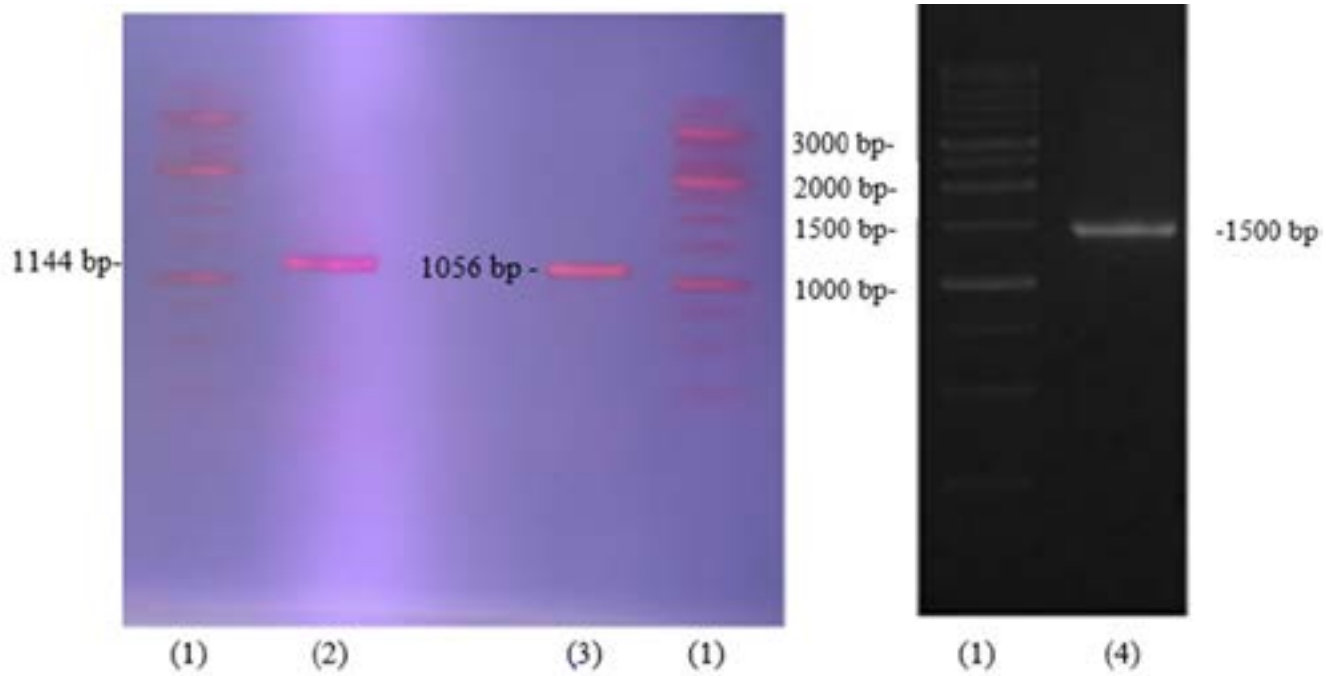


Figure 1. The electroforesis of 16s rRNA gene PCR products from selected actinomycetes. (1) marker 1 kb, (2) HJ4.5b, (3) P2b(b).3, (4) P2b(b).1

Table 1. The similarity of selected actinobacteria to GeneBank references strains based on 16S rRNA sequences

Isolate code	Closest relative species	Query cover	E value	Similarity (%)	Accession number
HJ4.5b	*Streptomyces jiujiangensis* strain JXJ 0074	100	0.0	99.04	NR.125706.1
P2b(b).3	*Streptomyces jeddahensis* strain G25	100	0.0	99.04	NR.156945.1
P2b(b).18	*Micromonospora* sp. strain SB	89	0.0	99.25	MG372012.1

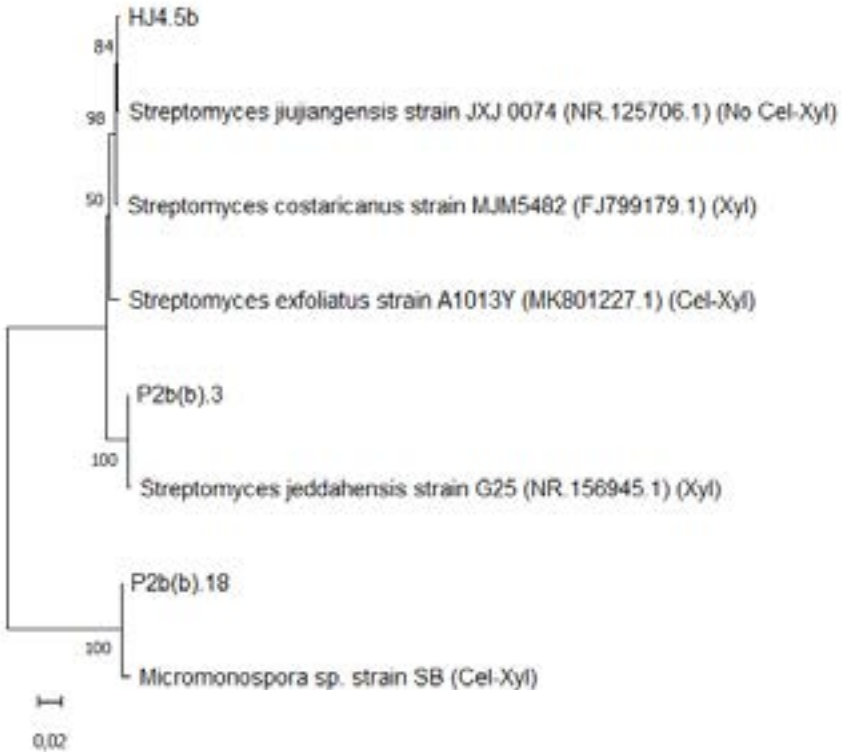


Figure 2. The construction of phylogenetic tree of HJ4.5b, P2b(b).3 and P2b(b).18 base on 16s rRNA gene sequence using 1,000x replication-bootstrap of the neighbor-joining method.

3.2. Fermentation of Coffee Cherries Using Selected Actinomycetes

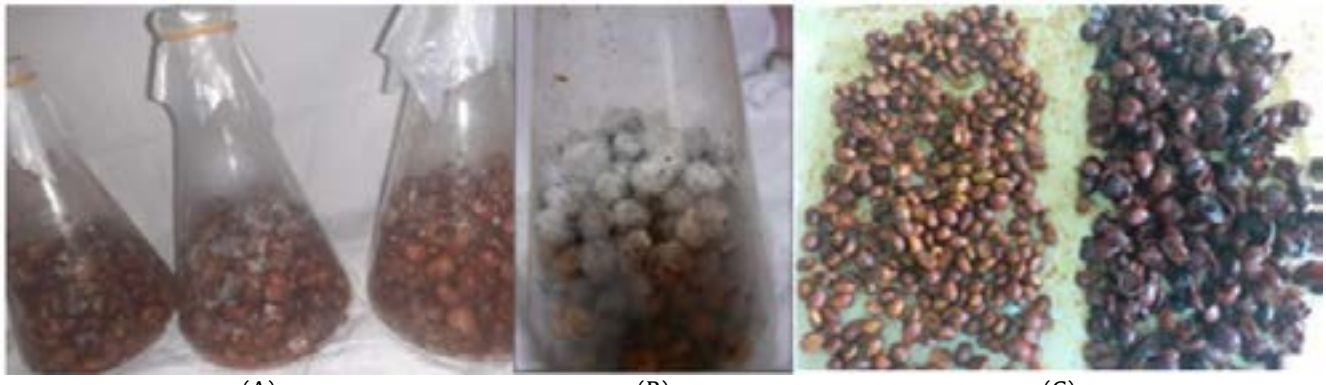
The fermentation process used the SSF method which was carried out for 9 days. On the first day of the fermentation process, the color changes from the coffee cherries to brown and the color intensity continues to increase until the fermentation ends (browning process) (Figure 3).

3.3. TFC and TPC

The TFC and TPC showed a significant difference ($p \leq 0.05$) between all treatments group (Table 2). The TFC in the treatment group using a starter agent showed an increase until the 9th day of fermentation. The highest percentage increase in TFC was indicated by the extract fermented using isolate P2b(b).3 reaching up to 295.54% (from 22.44 mgRE/g to 88.75 mgRE/g) on the 9th day of fermentation. The TFC in the group with starter culture isolates HJ4.5b, P2b(b).18, and control increased until fermentation on day 6 and decreased on day 9. However, in group with starter culture P2b(b).3 the TPC increased until the fermentation day 9. The highest of TPC was shown by the fermentation extract using P2b(b).3 isolate on the 9th day of fermentation with a value of 105.62 mgGAE/g, while the highest percentage increase was in the fermentation treatment with HJ4.5b reaching 193.00% at 6th day.

3.4. Antioxidant Activity

The lowest IC_{50} value (18.41 µl/ml) was obtained with isolate P2b(b).3 (Table 3) having an antioxidant activity index (AAI) value of 2.14 which was included in the very strong antioxidant activity category (AAI>2) (Table 4). Fermented extract using isolate P2b(b).18 as a starter showed the lowest IC_{50} value (24.30 µl/ml) on the 2nd day of fermentation (Table 3) with 1.62 of AAI (Table 4) (strong antioxidant activity category).



(A) (B) (C)

Figure 3. (A) Fermentation with P2b(b).3 culture on day 6, (B) fermentation with P2b(b).18 culture on day 9, (C) separation of beans and rind of fermented coffee fruit on day 6 with HJ4. 5b culture

Table 2. TFC and TPC of fermented exract of pulp coffee

Treatment	TFC and TPC of fermented coffee pulp extract on the day				
	0	1	2	6	9
	TFC* (mgRE/g)				
Control	26.56±1.02^{d}	27.74±1.77^{d}	42.46±2.04^{i}	49.65±2.15^{k}	47.29±0.89^{j}
HJ4.5b	20.20±0.41^{a}	24.56±0.61^{c}	29.98±0.41^{e}	32.10±0.20^{f}	34.57±0.54^{g}
P2b(b).3	22.44±0.71^{b}	52.24±0.20^{l}	59.66±0.20^{m}	75.56±0.20^{n}	88.75±0.82^{p}
P2b(b).18	36.34±0.41^{gh}	36.81±0.41^{h}	74.73±0.61^{n}	85.10±0.74^{o}	90.40±0.89^{p}
	TPC* (mgGAE/g)				
Control	44.77±0.67^{c}	51.41±2.80^{def}	54.28±3.74^{efg}	49.55±0.77^{d}	33.27±1.85^{a}
HJ4.5b	30.31±0.15^{a}	43.27±0.40^{c}	64.77±0.04^{h}	88.80±0.08^{l}	73.90±0.08^{ijk}
P2b(b).3	38.76±0.51^{b}	51.17±0.87^{de}	76.93±0.80^{k}	101.21±0.89^{m}	105.62±0.15^{n}
P2b(b).18	50.20±2.03^{d}	55.19±0.74^{fg}	75.35±2.21^{jk}	71.97±3.23^{ij}	71.38±1.78^{i}

*Each value is the mean value ± standard deviation. n = 3. Values with different superscript small letters are significantly different ($P \leq 0.05$). Control is the test group without the addition of starter culture

Table 3. The inhibitory concentration (IC_{50}) coffee pulp fermented extract using selected isolates

Treatment	IC_{50} (µg/ml) of fermented coffee pulp extract on the day-				
	0	1	2	6	9
Control	53.82±0.98^{k}	60.92±0.52^{l}	35.87±0.32^{gh}	29.48±0.67^{ef}	38.91±1.8^{1i}
HJ4.5b	75.70±1.13^{m}	61.05±1.29^{l}	38.47±1.65^{hi}	30.30±3.58^{f}	38.99±0.07^{i}
P2b(b).3	56.32±0.86^{k}	34.57±1.72^{g}	29.00±1.47^{def}	21.67±0.45^{b}	18.41±0.57^{a}
P2b(b).18	50.18±2.40^{j}	48.58±0.37^{j}	24.30±0.33^{c}	26.94±1.15^{d}	27.15±0.20^{de}

*Each value is the mean value ± standard deviation. n = 3. Values with different superscript small letters are significantly different (P≤0.05). Control is the test group without the addition of starter culture

Table 4. Antioxidant Activity Index (AAI) coffee pulp fermented extract using selected isolates

Treatment	AAI of fermented coffee pulp extract on the day-				
	0	1	2	6	9
Control	0.73±0.01^{cde}	0.65±0.01^{bc}	1.20±0.01^{gh}	1.34±0.03^{i}	1.01±0.05^{g}
HJ4.5b	0.52±0.01^{a}	0.65±0.01^{b}	1.03±0.05^{g}	1.31±0.15^{i}	1.01±0.01^{g}
P2b(b).3	0.70±0.01^{bcd}	1.14±0.06^{h}	1.36±0.07^{ij}	1.84±0.04^{m}	2.14±0.07^{n}
P2b(b).18	0.79±0.04^{def}	0.81±0.01^{ef}	1.62±0.02^{l}	1.46±0.06^{k}	1.45±0.01^{jk}

*Each value is the mean value ± standard deviation. n = 3. Values with different superscript small letters are significantly different (P≤0.05). Control is the test group without the addition of starter culture

4. Discussion

P2b (b).3 has similarities to *S. jeddahensis*. It was isolated from desert soil collected near Jeddah in Saudi Arabia and it was an aerobic xylan-degrading actinomyces and utilizes glucose, galactose, fructose, mannitol, rhamnose, arabinose, xylose, inositol, glycerol, lactose, maltose, cellobiose, sucrose, raffinose, and starch but not cellulose (Rottig *et al.* 2017). This report is in line with the results of this study. HJ4.5b has similarities to *S. Jiujiangensis* strain JXJ 0074. It was isolated from a soil sample collected from Jiangxi province, South China that was unable to utilize cellulose, D-cellobiose or D-xylose as a carbon source (Zhang *et al.* 2014). HJ4.5b in this study was able to utilize cellulose and D-cellubiose as carbon sources. Based on these differences, it is assumed that HJ4.5b is a different species, so that further research is needed. Among actinomycetes, *Micromonospora* (1.40%) is considered as the third dominant group of culturable soil actinomycetes after *Streptomyces* (95.43%) and *Nocardia* (1.98%) (Madigan *et al.* 2011) and it belongs to the rare actinomycetes. The ability of *Micromonospora* spp. to depolymerize the lignocellulosic biomass is relatively less studied compared to other genera from actinobacteria (Chen *et al.* 2020). The ability of *Micromonospora* spp. in degrading coffee pulp has just been reported in this study.

The phylogenetic tree construction exhibited that HJ4.5b and P2b(b).3 belonged to the cluster of *Streptomyces* but were in different branches of one another. They also showed different branch locations from the *Streptomyces* cluster which were known to be capable of producing cellulases and xylanases. Meanwhile, P2b(b).18 was in another cluster separate from the *Streptomyces* group cluster. The phylogenetic tree construction shows that P2b(b).18 is in a group with actinobacteria known as rare actinomycetes belonging to the genera *Micromonospora, Pseudarthrobacter, Frankia, Actinokineospora, Microbacterium, and Arthrobacter*.

During the fermentation process, the color changes from the coffee cherries to brown and the color intensity continues to increase until the fermentation ends (browning process). Enzymatic browning occurs in fruits which contain a lot of phenolic substrates. It is in line with the statement of Esquivel and Jimenez (2011) that the skin of the coffee cherries contains chlorogenic acid (42% of the total identified phenolic compounds). This is thought to be the cause of the change in color of the coffee cherries during fermentation. The growth of microorganisms can be seen clearly on the surface of the coffee fruit in the form of white colonies on the 6th day to the 9th day of fermentation. In addition, the presence of water vapor on the surface in the fermentation container and the smell of alcohol when the fermentation container was opened indicates that the isolates could grow well on the coffee cherries and the fermentation process was running well.

The increase in polyphenols during the fermentation process is thought to be due to the

breakdown of lignocellulose due to the action of cellulase and xylanase enzymes produced by Actinomycetes. Previous research conducted by Putri *et al.* (2019) reported that HJ4.5b and P2b(b).3 were isolates capable of producing cellulase and xylanase enzymes with hydrolytic index of 0.92 and 1.07 respectively by using coffee pulp powder as a nutrient source on solid media. While P2b(b).18 is an isolate that has the ability to produce cellulase and xylanase enzymes at the same time with a hydrolytic index of 0.62 on solid media with coffee pulp as a nutritional source (Putri *et al.* 2019). Cellulase enzyme activity HJ4.5b, xylanase enzyme activity P2b(b).3, and both cellulase and xylanase enzyme activity P2b(b).18 demonstrated the ability of the three isolates to degrade lignocellulosic biomass of coffee pulp (data not shown). Polyphenol in plants covalently bound to the cell wall and can be extracted by degrading lignocellulose components that bind them, such as lignin, protein, and carbohydrates (cellulose and hemicellulose) (Arellano-Gonzalez *et al.* 2011). Hydrolysis using enzymes is able to reduce the viscosity of the substrate, reduce attractive forces and the stability of interactions between molecules, increases cell permeability and porosity so that it will release of polyphenols and increase in the concentration of phenolic compounds from the extract (Cerda *et al.* 2013). It was in line with previous research, that both spontaneous and controlled fermentation by the work of microorganisms can increase the acquisition of polyphenols from coffee pulp extracts (Ameca *et al.* 2018; Arellano-Gonzalez *et al.* 2011; Garcia *et al.* 2015; Kurniawati *et al.* 2016; Lopez *et al.* 2013; Oktaviani *et al.* 2020). The fermentation process is also suspected to activate the action of endogenous pectinase and reduce pectin in the cell wall so that it will release covalently bound hydroxycinnamic acid and its derivatives (chlorogenic acid, caffeic acid, ferulic acid) (Verma *et al.* 2018). Yazdi *et al.* (2019) reported a study that the concentration of phenolic compounds increased after 2-4 h of enzymatic reaction by working of pectinase in pistachio pulp. The increase in polyphenols in the fermentation process is thought to be related to the presence of the cellulase enzyme which triggers the transglycosylation reaction. Ginko biloba leaf flavonol transglycosylation increased to 102% after being catalyzed with *Penicillium decumbens* cellulase (Chen *et al.* 2011).

Polyphenol content can affect antioxidant activity due to the presence of a hydroxyl group. Enzymatic activity may cause a change of phenolic compound structure and it also can increase the antioxidant activity (Oktaviani *et al.* 2020). Additionally, the radical scavenging and chelating activity of a flavonoid are influenced by the setting of its chemical structure that is based on the flavan nucleus, the number, position, and types of substitutios. Fermentation of coffee pulp using indigenous lactic acid bacteria as a starter can increase the antioxidant activity up to 30% higher than the fresh coffee pulp (Oktaviani *et al.* 2020). The fermented extract with isolate P2b(b).3 experienced a higher total flavonoid and phenolic content reaching 2.95 and 1.72 times respectively compared to fermentation on day 0. Antioxidant activity had a very significant correlation with the total content of polyphenol. In this study the increase in polyphenols during coffee pulp fermentation by selected actinomycetes could occur due to several factors that we can reveal: The occurrence of degradation of lignocellulosic biomass by cellulolytic and xylanolytic actinomycetes, degradation of pectin components, production of secondary metabolites by actinomycetes during the fermentation process, and reactions that may occur during fermentation. Plant cells or microorganisms are able to produce pectinase enzymes naturally and degrade pectin in the cell wall. Hydroxycinnamic acid and its derivative compounds (chlorogenic acid, caffeic acid, ferulic acid) are covalently bound to dicotyledon plant pectins, so that by reducing pectin, these polyphenols will be released and increase their extraction (Verma *et al.* 2018).

In this study, total flavonoid levels increased significantly on the 9th day of fermentation. It is suspected that on day 9 secondary metabolites were produced by selected actinomycetes due to a decrease in glucose production which activated secondary metabolism. The production of secondary metabolites by actinomycetes plays a role in adaptation and protection against changes in environmental conditions such as shortages and depletion of nutrients, formation of harmful compounds during the fermentation process. Another thing that strengthens the production of secondary metabolites by selected actinomycetes is their ability to produce pigments on ISP4 media, where HJ4.5b and P2b(b).3 produce yellowish-white and grayish-white pigments, respectively.

The ability of selected actinomycetes to produce secondary metabolites during the fermentation process requires further research.

In conclusion, the fermentation coffee cherries using three indigenous actinomycetes in this study significantly increased TFC and TPC and affected the antioxidant activity of fermented extract of coffee pulp. Thus, coffee pulp, which has only been a wasted waste, can be used as a source of active polyphenol antioxidant compounds which have high economic value in the pharmaceutical and food industries. For further research need to analyze the changes in polyphenol compounds that occur during the fermentation process and their relationship with pharmacological activity.

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References

Adam, A., Hakim, M.N., Oktaviani, L., Inderaja, B.M., Manurung, R., Putra, R.E., Abduh, M.Y., 2020. Techno-economic evaluation for integrated cultivation of coffee and stingless bees in West Java, Indonesia. *Biol. Nat. Resour. Eng. J.* 3, 28-36.

Ameca, G.M., Ortega Cerrilla, M.E., Cordoba, P.Z., 2018. Chemical composition and antioxidant capacity of coffee pulp. *Ciencia e Agrotecnologia.* 42, 307-313. https://doi.org/10.1590/1413-70542018423000818

Arellano-Gonzalez, M.A., Ramirez-Coronel, M.A., Torres-Mancera, M.T., Perez-Morales, G.G., Saucedo-Castaneda, G., 2011. Antioxidant activity of fermented and nonfermented coffee (*Coffea arabica*) pulp extracts. *Food Technol. Biotechnol.* 49, 374-378.

Bruce, K.D., Hiorns, W.D., Hobman, J.L., Osborn, A.M., Strike, P., Ritchie, D.A., 1992. Amplification of DNA from native populations of soil bacteria by using the polymerase chain reaction. *Appl. Environ. Microb.* 58, 3413-3416. https://doi.org/10.1128/aem.58.10.3413-3416.1992

Cerda, A., Martínez, M.E., Soto, C., Poirrier, P., Perez-Correa, J.R., Vergara-Salinas, J.R., Zuniga, M.E., 2013. The enhancement of antioxidant compounds extracted from Thymus vulgaris using enzymes and the effect of extracting solvent. *Food. Chem.* 139, 138-143. https://doi.org/10.1016/j.foodchem.2012.12.044

Chen, S.J, Lam, M.Q, Thevarajoo, S., Manan, F.A., Yahya, A., Chong, C.S., 2020. Genome analysis of cellulose and hemicellulose degrading *Micromonospora* sp. CP22. *3. Biotech.* 10, 160-170. https://doi.org/10.1007/s13205-020-2148-z

Chen, S., Xing, X.H., Huang, J., Xu, M.S., 2011. Enzyme-assisted extraction of flavonoids from *Ginkgo biloba* leaves: improvement effect of flavonol transglycosylation catalyzed by *Penicillium decumbens* cellulase. *Enzyme. Microb. Technol.* 48, 100-105. https://doi.org/10.1016/j.enzmictec.2010.09.017

Esquivel, P., Jimenez, V. ., 2011. Functional properties of coffee and coffee by-products. *Food. Res. Int.* 46, 488-495. https://doi.org/10.1016/j.foodres.2011.05.028

Garcia, L.R.P., Biasetto, C.R., Araujo, A.R., del Bianchi, V.L., 2015. Enhanced extraction of phenolic compounds from coffee industrys residues through solid state fermentation by *Penicillium purpurogenum. Food. Sci. Technol.* 35, 704711. https://doi.org/10.1590/1678-457X.6834

Jamil, A.S., 2019. Daya saing ekspor kopi Indonesia di pasar global. *Agriekonomika.* 8, 26-35. https://doi.org/10.21107/agriekonomika.v8i1.4924

Janatiningrum, I., Solihin, D.D, Meryandini, A., Lestari, Y., 2018. Comparative study on the diversity of endophytic actinobacteria communities from *Ficus deltoidea* using metagenomic and culture-dependent approaches. *Biodiversitas.* 19, 1514-1520. https://doi.org/10.13057/biodiv/d190443

Kurniawati, N., Meryandini, A., Sunarti, T.C., 2016. Introduction of actinomycetes starter on coffee fruits fermentation to enhance quality of coffee pulp. *Emir. J. Food. Agric.* 28, 188-195. https://doi.org/10.9755/ejfa.2015-05-192

Lopez, T., Prado-Barragan, A., Nevarez-Moorillon, G.V., Conteras, J.C., Rodriguez, R., Aguilar, C.N., 2013. Incremento dela capacidad antioxidante de extratos de pulpa de cafe por fermentacion lactica en medio solido. *CyTA- J. Food*. 11, 359-365. https://doi.org/10.1080/19476337.2013.773563

Madaan, R., Kumar, S., 2011. Estimation of total phenols and flavonoids in extract of *Actae spicata* roots and antioxidant activity studies. *Indian. J. Pharm. Sci.* 73, 666-669. https://doi.org/10.4103/0250-474X.100242

Madigan, M.T., Martinko, J.M., Parker, J., 2011. *Biology of microorganisms.* twelfth ed. Prentice Hall International, New York.

Martina, K., Jan, K., Tamas, F., Ladislav, C., Marek, O., Genevieve, L.G., 2008. Development of a 16S rRNA gene-based prototype microarray for the detection of selected actinomycetes genera. *Anto. Leeuw. Intl. J. G.* 94, 439-453. https://doi.org/10.1007/s10482-008-9261-z

Oktaviani, L., Astuti, D.I., Rosmiati, M., Abduh, M.Y., 2020. Fermentation of coffee pulp using indigenous lactic acid bacteria with simultaneous aeration to produce cascara with a high antioxidant activity. *Heliyon.* 6, e04462. https://doi.org/10.1016/j.heliyon.2020.e04462

Orozco, A.L., Perez, M.I., Guevara, O., Rodriguez, J., Hernandez, M., Gonzalez-Vila, 2008. Biotechnologic al enhancement of coffee pulp residues by solid-state fermentation with *Streptomyces*. Py-GC/MS analysis. *J. Anal. Appl. Pyrol.* 81:247-252. https://doi.org/10.1016/j.jaap.2007.12.002

Payal, D., Renu, S., and Monisha, K., 2014. Isolation and screening of cellulolytic actinomyctes from diverse habitats. *IJBR.* 5, 438-451.

Putri, E., Rukayadi, Y., Sunarti, T.C., Meryandini A., 2019. Cellulolytic and xylanolytic actinomycetes selection to degrade lignocellulosic biomass of robusta coffee pulp (*Coffea canephora*). *IOP Conf. Ser.: Earth Environ. Sci.* 299, 012014. https://doi.org/10.1088/1755-1315/299/1/012014

Rottig, A., Atasayar, E., Kolthoff, J.P., Sproer, C., Schumann, P., Schauer, J., Steinbuchel, A., 2017. *Streptomyces jeddahensis* sp. nov., an oleaginous bacterium isolated from desert soil. *Int. J. Syst. Evol. Microbial.* 67, 1676-1682. https://doi.org/10.1099/ijsem.0.001839

Saini, A., Anggarwal, N., Sharma, A., and Yadav, A., 2015. Actinomycetes: a source of lignocellulolytic enzymes. *Enzyme Research.* 2015, 1-15. https://doi.org/10.1155/2015/279381

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mole. Biol. Evol.* 4, 406-425.

Santos, da Silveira, J., Durand, N., Lacour, S., Belleville, M. P., Perez, A., Loiseau, G., Dornier, M., 2019. Solid-state fermentation as a sustainable method for coffee pulp treatment and production of an extract rich in chlorogenic acids. *Food Bioprod Process*. 115, 175-184. https://doi.org/10.1016/j.fbp.2019.04.001

Scherer, R., Godoy, H.T., 2009. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picryl-hydrazyl method. *Food. Chem.* 112, 654-658. https://doi.org/10.1016/j.foodchem.2008.06.026

Vasic, S.M, Stefanovic, O.D, Licina, B.Z, Radojevic, I.D, Comic, L.R., 2012. Biological activities of extracts from cultivated granadilla passiflora alata. *EXCLI. J*. 11, 208-218.

Verma, H., Naroliya, L., Jadaun, J.S., 2018. Pectinase: a useful tool in fruit processing industries. *Nutr. Food Sci. Int. J.* 5, 1-4. https://doi.org/10.19080/NFSIJ.2018.05.555673

Woldesenbet, A.G., Woldeyes, B., Chandravanshi, B.S., 2016. Bio-ethanol production from wet coffee processing waste in Ethiopia. *SpringerPlus*. 5, 1-7. https://doi.org/10.1186/s40064-016-3600-8

Yazdi, A.P.G., Barzegar, M., Sahari, M.A., Ahmadi Gavlighi, H., 2019. Optimization of the enzyme-assisted aqueous extraction of phenolic compounds from pistachio green hull. *Food. Sci. Nutr.* 7, 356-366. https://doi.org/10.1002/fsn3.900

Zhang, B.H., Cheng, J., Li, L., Zhang, Y.G., Wang, H.F., Li, H.Q., Yang, J.Y, Li, W.J., 2014. *Streptomyces jiujiangensis* sp. nov., isolated from soil South China. *Antonic van leeuwenhoek.* 105, 763-770. https://doi.org/10.1007/s10482-014-0132-5