Cellulolytic and Xylanolytic Actinomycetes selection to degrade Lignocellulosic biomass of Robusta coffee pulp (*Coffea canephora*)

IOP Conf. Series: Earth and Environmental Science **299** (2019) 012014 doi:10.1088/1755-1315/299/1/012014

Cellulolytic and Xylanolytic Actinomycetes selection to degrade Lignocellulosic biomass of Robusta coffee pulp (*Coffea canephora*)

E Putri[1], Y Rukayadi[2], T C Sunarti[3] and A Meryandini[4*]

[1]Study Program of Microbiology, IPB University, Bogor 16680, Indonesia
[2]Department of Food Science, Faculty of Food Science and Technology, University Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia
[3]Department of Agroindustrial Technology, IPB University, Bogor 16680, Indonesia
[4]Department of Biology and Research Center for Bioresources and Biotechnology (RCBIO), IPB University, Bogor, 16680, Indonesia

*E-mail: ameryandini@apps.ipb.ac.id

Abstract. Actinomycetes are bacteria that have a high ability to produce hydrolytic enzymes that can be applied to degrade coffee pulp which contains lignocellulose components. Coffee pulp is residues from a coffee industry that can be used as substrates in the solid state fermentation process. In this study, cellulase and xylanase activity from actinomycetes isolates were investigated. Screening of these enzymes activity was using agar medium supplemented with 1% carboxymethylcellulose (CMC) and xylan from beechwood as substrate. Production of clear zones surrounding isolates on CMC and xylan agar medium was considered as indicative of extracellular cellulase and xylanase activity. The size of a transparent zone diameter was considered as proportional to the level of cellulase and xylanase production. Congo red 0,1% was used for staining. Selected actinomycetes isolates were continued to growth investigation on agar medium supplemented with 1% coffee pulp. The clear zone formed around isolates indicated that the isolate had the ability to degrade the lignocellulose component in the coffee pulp. The results show that of the 99 actinomycetes isolates tested on agar medium supplemented with 0.1% CMC and xylan, 16 isolates showed cellulase activity (cellulase index ≥ 2), 20 isolates showed xylanase activity (xylanase index ≥ 1), and 5 isolates showed both activities. Ten isolates have the ability to grow and produce a clear zone on the coffee pulp agar medium with cellulase and xylanase activity. Among these ten tested isolates; HJ4.5b, P2b(b).3, P2b(b).4, and P2b(b).18 are the most potential isolates that can be continued for further research on fermentation coffee cherries.

1. Introduction

Actinomycetes are filamentous gram-positive bacteria that have a high ability to produce various bioactive compounds. Besides producing antibiotic compounds actinomycetes are also capable to produce various enzymes that are very useful to be applied in the biotechnology process. Actinomycetes have a pseudo mycelium which is able to penetrate into the substrate and secrete extracellular hydrolytic enzymes that are able to degrade lignocellulosic biomass. The lignocellulolytic enzyme is one of the potential enzymes that can be widely applied in lignocellulose-based industries [1].

Lignocellulosic biomass is the most abundant source of renewable organic material in nature (more than 200 billion tons annually) that is readily available to be converted into biofuels material and other value-added products that have high economic value (enzyme industry, organic acids, pharmaceuticals, chemical commodities, and food) [2]. Agricultural by-products are one of a considerable amount of lignocellulosic biomass which is comprised of about 10-25% lignin, 20-30% hemicellulose, 40-50% cellulose as the main constituents of plant cell walls and extractive component [3][4]. Cellulose is a skeletal compound that makes up 40-50% of the wood in the form of microfibril cellulose, while hemicellulose is a matrix compound that consists of repeated polymers of pentoses and hexoses. Lignin contains three aromatic alcohols (coniferyl alcohol, sinapyl alcohol, and pcoumaryl alcohol) produced through a biosynthetic process and forms a protective seal around the other two components i.e., cellulose and hemicelluloses [3].

Coffee is a very popular agricultural commodity and the second most important trading commodity in the world market after petroleum. Indonesia is one of the largest producers and exporters of coffee in the world. Indonesian coffee production on a land area of 1 227 787 ha reaches 637 539 tons per year [5]. The coffee industry generates high amounts of residues since coffee is one of the most consumed beverages in the world [6][7]. Coffee pulp is the main waste in the processing of coffee beans which reaches 40-50% of the wet weight of coffee cherries. The coffee pulp contains protein, fat, carbohydrates and minerals such as potassium. Lignocellulosic biomass of coffee pulp contains 49% cellulose, 24.5% hemicellulose and 7.63% lignin [8]. Thus the coffee pulp contains complete nutrients that can be utilized by actinomycetes as a source of carbon and nitrogen for its growth. Coffee pulp also contains bioactive compounds of flavonoid polyphenols (tannins, flavonols, catechins, anthocyanins) and phenolic acids (hydroxycinnamic acid, caffeic acid, and chlorogenic acid) [9,7].

Coffee cherries must be processed in several stages so that it can be consumed in the form of coffee bean powder. The processing of coffee cherries can be done by wet, semi-dry and dry methods [10]. Coffee cherries that are processed with wet methods can generally produce coffee beans with better quality [11] [6]. The wet method undergoes a spontaneous fermentation process involving various kinds of complex microorganisms (bacteria, mold, yeast) so that it will produce a variety of qualities of flavor and aroma of coffee beans [10]. To overcome this, selective bacterial and yeast cultures were added. Specific microorganisms selected for starter culture during the coffee fermentation process are important to improve the quality of fermentation, organoleptic and sensory coffee drinks and shorten processing time [12] [10]. Based on Kurniawati et al. (2016) [13], for the first time reported the introduction of actinomycetes consortia on fermentation of coffee pulp can also enhance the yield and quality of coffee pulp polyphenol extracts. The degradation of lignocellulosic biomass coffee pulp can release the polyphenol compounds that are covalently bound to the cell wall [14]. Actinomycetes can produce extracellular hydrolytic enzymes that have cellulolytic and xylanolytic activities. *Streptomyces lividan* and *Streptomyces flavogriseus* produce cellulase [15]. Xylanase was isolated and characteristic from Streptomyces sp. E-86 [16]. Astuti (2011) [17] and Apriyani (2012) [18] stated that *Streptomyces exfoliatus* 42 has the ability to produce endoglucanase, exoglucanase, and xylanase.

There is a lot of research has been conducted on cellulolytic and xylanolytic microorganisms which focused on the utilization of agricultural waste as substrates through solid-state fermentation (SSF). However, relatively fewer studies focused on the degradation of agricultural by-products of lignocellulosic biomass by the action of actinomycetes. In this study, cellulase and xylanase activities from actinomycetes isolates were investigated to degrade lignocellulosic biomass of robusta coffee pulp (*Coffea canephora*).

2. Material

The materials used in this study include red robusta coffee cherries, actinomycetes isolates. Chemicals used include ISP4 medium which contained [19]: 1.0 g soluble starch, 0.1 g K2HPO4, 0.1 g NaCl, 0.2 g (NH4) 2SO4, 0.2 CaCO3, Trace Salt Solution (0.1 mg FeSO4.7H2O, 0.1 mg MnCl2. 7H2O, 0.1 mg ZnSO4. 7H2O), 0.1 g MgSO4. 7H2O, 2 g agar (per 100 mL), Carboxymetyl Cellulose (CMC), ethanol

70%, ethanol 96%, H2SO4 72%, acetone, aquades, FeCl3, 48% HBr solution, phenol, Neutral Detergent Solvent (NDS), Acid Detergent Solvent (ADS), congo red 1%. 0.2 M NaCl.

3. Methods

3.1. Coffee pulp substrate preparation

3.1.1. Substrate raw material collection. Robusta coffee cherries collected from the smallholding plantation of Pagar Alam, South Sumatra. Coffee cherries picked directly with the coffee fruits character that was ripe red. The coffee cherries were then washed with running water and separated from the skin and seeds (pulped). Coffee pulp was dried and then ground to 100 mesh powder.

3.1.2. Characterization of substrate raw materials. Coffee pulp as a substrate that has been dried and milled to form a powder with a size of 100 mesh, then carried out chemical analysis in accordance with the method of proximate analysis includes water content, ash content, protein, fat, carbohydrates [20], determined fiber content according to the method of Van Soest et al. (1963) [21]. The fiber content of cellulose, hemicellulose, and lignin.

3.2. Selection of cellulolytic and xylanolytic actinomycetes Isolates

3.2.1. Preliminary tests for cellulolytic and xylanolytic capacities. Actinomycetes isolates were selected from the collection of Animal Biotechnology and Biomedical Laboratory, Center for Life Science & Biotechnology, IPB University, Bogor - Indonesia. There are 99 actinomycetes isolates were screened for their cellulase and xylanase production. Cellulolytic and xylanolytic activities were assayed first through qualitative tests by growth on solid ISP medium No. 4 containing CMC 1% (screening of cellulase) [34] and beechwood xylan 1% (screening of xylanase) [36]. Cellulose and xylan degradation was indicated by the formation of a cleared zone of hydrolysis around the colonies which was measured to select cellulase and xylanase producing isolates [34]. The clear zone formed after adding a 0,1% congo red solution in the medium was measured to determine the index activity value of cellulase and xylanase enzymes. This clear zone was measured for subsequent calculation of the enzymatic index (EI) using the expression [35]:

$$\mathrm{EI} = \frac{\text{Diameter of hydrolysis zone}}{\text{diameter of the colony}} \quad (1)$$

The Isolates that showed cellulase enzyme index ≥ 2 and xylanase enzyme index ≥ 1 were considered to be potential producers of cellulases and xylanases [35]. These potential isolates then continued to the test growth and formation of clear zones on the coffee pulp substrate.

3.2.2. Test the growth and formation of clear zones on coffee pulp substrate. Isolates were rejuvenated on ISP4 media and then incubated for 48 hours at room temperature. Isolates that were 3 days old were then grown on ISP4 media but starch was replaced with a 1% dried coffee substrate. One cockborer isolate was transferred to solid dried coffee pulp substrate media. Triple experiments were carried out. Then observed the growth of isolates every 24 hours for 48 hours by measuring the diameter of the isolates formed in the media. On the 2nd day, the media was given a 0,1% congo red solution. After 15 minutes the media was rinsed with 0.2 M NaCl. Cellulase and Xylanase production was indicated by the appearance of a pale halo with orange edges, indicative of areas of hydrolysis. Then the diameter of the pale halo formed was measured by comparing the diameter of the hydrolysis zone with a diameter of the colony [35].

4. Results and Discussion

4.1. Coffee Pulp Substrate Preparation

4.1.1. Substrate Raw Material Collection. Robusta coffee was taken from smallholder plantations on February 18, 2018, in the Trans village of Pagar Alam city, South Sumatra. Coffee cherries were picked directly which have character was ripe red. From 3500 g of ripe coffee fruits, 1600 g of fresh coffee pulp was obtained. Coffee pulp was dried for 4 weeks and yield 280 g of dry coffee pulp. The dried coffee pulp was then mashed and filtered using 100 mesh so that 240 g of coffee pulp powder was obtained. Coffee pulp powder was then stored in a dark glass container for later use in subsequent studies.

The degree of maturation is one of the parameters that determine the final quality of coffee drinks. During maturation, the ovary usually develops into a double – seed stone fruit which is round to elongated and pointed shape, while the fruit of robusta coffee is slightly smaller and rounder. During maturation, the coffee cherry changes clours-from the initial green and immediately turns yellow, and the ripe fruit finally shines in bright red. The interior of the coffee cherry shows: the two seeds, the actual coffee beans are surrounded by whitish yellow, sweet pulp and facing each other with their flat sides which have the typical longitudinal groove. The two coffee beans are also surrounded by the seed skin called silver skin, a thin mucous layer as well as the pale yellow parchment. In this condition, the matured coffee cherries can now be harvested and processed [22] [23]. Tarigan and Towaha (2017) [24] reported that fruit maturity affects the physicochemical characters and fermentation time of robusta coffee cherries. Coffee cherries that are harvested in red produce fermented seeds with more moisture high and the number of defective seeds is lower than seeds harvested in reddish yellow. Fruit coffee harvested in reddish yellow, then fermented for 36 hours and roasted for 13 minutes produce relatively high levels of fat and caffeine higher than the red coffee cherries.

In perfectly ripe coffee fruit, various chemical components are formed which are very necessary when producing high-quality roasting coffee beans. There is a production of mucilage that contains fermented carbohydrates for the fermentation process. In addition, pectin substances are formed around 35% of the dry weight of mucilage, including polygalacturonic acid which is bound as a glycosidic link needed to produce enzymes. Various minerals such as Ca, K and P also present in mature red of coffee cherries [22]. Additionally, another important thing that forms during the maturation of coffee cherries is the production of extractive substances such as organic acids, polyphenols, and alkaloids compounds. Chlorogenic acids are present in high concentrations in the green coffee seed of maturity fruits (up to 14 %), have a marked influence in determining the coffee quality and play an important role in the formation of coffee flavor [25] [26] [27]

4.1.2. Characterization of Substrate Raw Materials. The chemical analysis showed that dried coffee pulp contains protein, lipid, carbohydrate, and fiber as listed in the table below:

Table 1. The chemical composition of dried coffee pulp

Chemical Compositions	Composition (% DW) This research	Kurniawati et al 2016	Braham &Bressani 1979
Protein	11.37	3.9	11.2
Lipid	2.64	1.51	2.3
Moisture	10.08	-	12.6
Ash	9.59	3.43	8.3
Carbohydrate	25.11	28.5	48.59
Fiber			
Cellulosa	18.19	53.42	17.7
Hemicellulose	5.46	24.10	2.3
Lignin	17.56	6.1	17.5

Figure 1. Red of maturity coffee cherries (a), coffee fruit pulped (b), dried coffee pulp powder (c) (personal collection).

According to Rachman (1999) [28], bacteria need protein as one of the organic nitrogen in its growth. The results of the analysis of coffee pulp provide information that coffee pulp is a material that provides a nitrogen source and carbon source for microbial growth. This is because the coffee pulp is a complex component consisting of crude fiber, protein, lipid, carbohydrates, total sugar, and reducing sugars. Thus the coffee pulp can be used as a single substrate for the growth of actinomycetes without the addition of other components as nutrients in the cultivation process. The substrate with high carbohydrate and protein content has the potential to be used in bioprocess [29]. Based on this result, robusta coffee pulp from Pagar Alam, South Sumatra perhaps can be degraded by actinomycetes that have the ability to produce cellulolytic and xylanolytic enzymes.

4.2. Selection of Cellulolytic and Xylanolytic Actinomycetes

Selected cellulase and xylanase activities from 99 actinomycetes isolates were isolated from soil of Bukit Dua Belas forest land, Jambi. Forty-one isolates had cellulase and xylanase activities on CMC and Xylan substrate with ISP medium no.4. There were 16 isolates showed cellulase index ≥2, 20 isolates with xylanase index ≥1 and 5 isolates showed both cellulase and xylanase index ≥1 (Table 2).

Screening for extracellular cellulase production by bacteria and fungi is often done on agar plates containing CMC and xylan as substrate [30] [36]. The selection of strain that can efficiently degrade polysaccharides such as cellulose, xylan, and amylose can be done by measuring the diameter of the halo zone [38] and the enzymatic index can be used as a simple and rapid methodology to select strain within the same genus that have potential for the production of enzymes [38]. The detection of the cellulolytic activity in these cases is achieved by staining or precipitation of undigested CMC in plate regions that were not exposed to cellulolytic activity, while areas exposed to cellulase give clear halos surrounding the source of the enzyme. This method is popular because large numbers of samples can be monitored and compared simultaneously and quickly [31,33]. Over the decades, congo red is one of the dyes which has been introduced for this differential staining, besides gram's iodine. Congo red only remains attached to regions where there is β-1,4-D-glycosidic linkage. The hydrolysis of cellulose and xylan by working of cellulolytic and xylanolytic enzymes produced halo region which is directly related to the action of enzymes [37]

Table 2. Isolates showed cellulase and xylanase activities

No	Isolate Code		
	CI ≥ 2	XI ≥ 1	CI ≥2 and XI ≥1
1	OM4CW(c).14	P22(5b)	P2b(b).15
2	OM4CW(c).19	HJ4 (2)	P2b(b).18
3	OM2RH(e).2	HJ4(5a)	OM1CH(D).12
4	OM2RH(e).3 (a)	HJ4 (7)	HR4(2)
5	OM2RH(e).3 (b)	HR4 (1)	HR4(5)
6	P22(1)	OM4CW(4a)	
7	P22(2)	OM4RH (2)	
8	P22(4)	P4 (6)	
9	P22(6)	P4 (7)	
10	HJ4(2a)	BJ2 (a).1	
11	HJ4(3a)	BJ2 (a).11	
12	HJ4(3b)	P2b(b).3	
13	HJ4(5b)	P2b(b).4	
14	HJ4(6)	P2b(b).6	
15	HR4(2)	OM4CW(C).2	
16	OM4CW(3)	OM1CH(D).9	
17		OM1CH(D).11	
18		OM1CH(D).16	
19		OM2RH(e).1	
20		BO5(c).22	

CI = Cellulase Index
XI = Xylanase Index

A lot of previous studies reported screening of xylanase and cellulase actinomycetes base on the congo red plate method. Pushpendra S et al (2013) [36] reported 22 isolates of actinomycetes showed xylanase activity. Sixty-nine Alkalo-tolerant actinomycetes were selected as xylanase producers with enzymatic index ≥ 1.5 from the soil samples collected from various sites in around Delhi [40]. Lamia MH et al (2017) [41] conducted a preliminary qualitative analysis cellulolytic and xylanolytic

actinobacteria by carboxymethyl cellulose (CMC) and xylan-containing agar plates methods. This study reported 82 actinobacteria isolates showed promising enzymes activities (CMCase, xylanase) with diameter clear zones varying between 19 mm and 53 mm. Five cellulolytic actinobacteria isolated from diverse habitats in Delhi, India have promising enzyme activity with diameter hydrolysis zones between 17-33 mm [34]

4.3. Test of actinomycetes cellulase and xylanase activities on agar coffee pulp substrate medium

Forty-one actinomycetes isolates which had cellulase and xylanase activities on CMC and Xylan substrate were grown on the coffee pulp substrate medium. A total of 10 isolates have the ability to grow on coffee pulp medium (table 3). These ten isolates have different growth abilities as measured by the diameter of the isolate and the ability to produce clear zones around it. Growing isolates in the coffee medium showed that the isolates were able to use lignocellulolytic components on the substrate as a carbon source.

Table 3. Actinomycetes growth and clear zone formed on coffee pulp substrate medium

No	Isolates	Growth	Clear zone Index	No	Isolates	Growth	Clear zone Index
1	BJ2(a).1	-	-	22	P22.1	-	-
2	BJ2(a).11	-	-	23	P22.2	-	-
3	P2b(b).3	++	1,07	24	P22.4	-	-
4	P2b(b).4	++	1,67	25	P22.6	+	0,38
5	P2b(b).6	-	-	26	P22.5b	-	-
6	P2b(b).15	-	-	27	HJ4.2	-	-
7	P2b(b).18	++	0,62	28	HJ4.2a	-	-
8	OM4CW(c).2	-	-	29	HJ4.3a	-	-
9	OM4CW(c).14	-	-	30	HJ4.3b	+	0,36
10	OM4CW(c).19	-	-	31	HJ4.5a	-	-
11	OM1CH(D).9	-	-	32	HJ4.5b	++	0,92
12	OM1CH(D).11	-	-	33	HJ4.6	+	0,38
13	OM1CH(D).12	+	0,63	34	HJ4.7	-	-
14	OM1CH(D).16	-	-	35	HR4.1	-	-
15	OM2RH(e) .1	-	-	36	HR4.2	-	-
16	OM2RH(e) .2	-	-	37	HR4.5	-	-
17	OM2RH(e).3(a)	-	-	38	HR4.6	+	0,43
18	OM2RH(e).3(b)	-	-	39	OM4CW.3	-	-
19	BO5(c).22	+	0,45	40	OM4CW.4a	-	-
20	P4.6	-	-	41	OM4RH.2	-	-
21	P4.7	-	-				

++ isolate diameter ≥ 9 mm
+ isolate diameter < 9 mm

All isolates showed different ability in utilizing carbon sources on coffee pulp medium. P2b (b).3 isolate has the best growth ability on the coffee pulp substrate medium with an isolate diameter of 9.67 mm. The clear zone formed is quite clear with an enzymatic index of 1.07. While P2b (b).4 isolates have a higher index of 1.67 but the clear zone is not so clear compared to P2b (b).3 isolate.

P2b (b).3 and P2b (b).4 isolates are xylanolytic actinomycetes that have the best ability to produce xylanase. Isolates that are able to produce xylanase will form a clear zone on media containing xylan which is a component of hemicellulose. After 48 hours incubation and 0,1% congo red staining, the clear zone formed showed that the isolate was able to degrade xylan substrate into a simpler form, for example, xilo-oligosaccharides (XOS). Congo red can be used to clarify the clear zone formed. Congo

red works by binding to polysaccharides in β-1-4 glycoside bonds. When xylan has hydrolyzed to XOS, congo red does not completely bind oligosaccharides so the red color will appear faded. Flushing with NaCl will dissolve congo red so that a clear zone will be seen [32]. The more xylanase produced by xylanolytic bacteria, the clearer zones that are formed will be more widespread because of more and more degraded xylan.

HJ4.5b isolate is the best cellulolytic actinomycetes that produce cellulase enzymes. The clear zone formed on the coffee pulp substrate medium showed that this isolate was able to hydrolyze cellulose into its monomers such as cellobiose and glucose. Coloring with congo red will clarify the clear zone that is formed. The enzymes involved in the breakdown of cellulose are endoglucanases (Egs) that randomly cut the chains of cellulose mainly at the level of the amorphous zones generating new chain ends; cellobiohydrolases (CBHs) or exoglucanases which act possessively on the free ends of cellulose chain releasing cellobiose; β-glucosidase (BGLs) which hydrolyze cellobiose and soluble cellodextrins into glucose [39]

P2b (b).18 and OM1CH (D).12 isolates are two actinomycetes which have the ability to hydrolyze both cellulose (CMC) and hemicellulose (xylan). On the coffee pulp substrate medium, these two isolates showed good growth and showed clear zones after congo red coloring.

At the present time, there is no citation in the scientific literature describing the use of dried coffee pulp as a substrate on agar medium for screening cellulase and xylanase activities by the action of actinobacteria. In our study, dried coffee pulp used contains cellulose, hemicellulose, and lignin. Cellulose and hemicellulose were hydrolyzed by actinobacteria that are capable to produce cellulase and xylanase. This was indicated by the formation of clear zones around the isolate after staining 0,1 % congo red solution (figure 2-5). Lignin as a part of lignocellulosic biomass in this coffee pulp was thought not to be hydrolyzed.

Lignin is a complex recalcitrant polymer that can be degraded by lignin peroxidase, Mangan peroxidase, and laccase. These enzymes work by oxidizing phenolic aromatic polymer compounds forming lignin [42]. During the first steps of the reaction, phenol is degraded to dihydroxylated rings (catechol, , and hydroquinone). These aromatic intermediates generate higher colored compounds. On the other hand, the dihydroxylated rings can react with their own quinones to generate charge-transfer complexes (quinhydrone), compounds that take on a dark color at low concentrations [43]. In this study, there was no dark brown or higher color formed around isolates on agar coffee pulp medium (figure 2-5), so it indicated isolates did not produce ligninase enzymes.

Figure 2. P2b(b).3 isolate

Figure 3. P2b(b).4 isolate

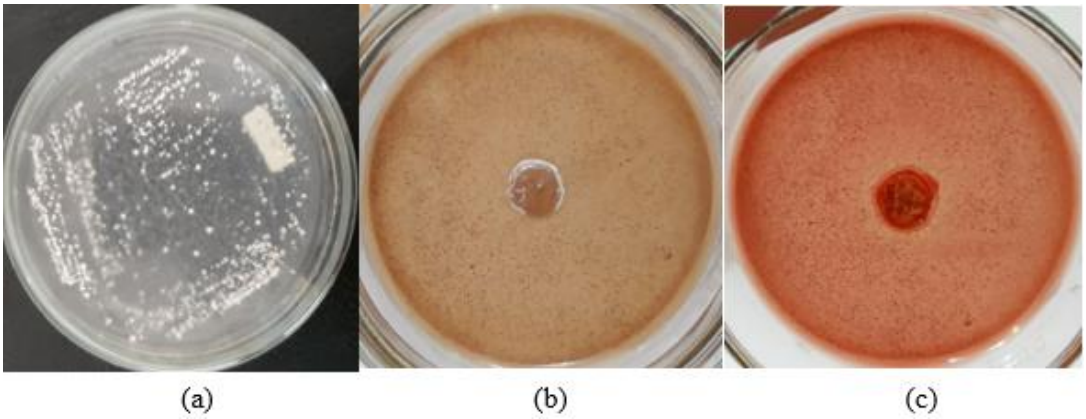

Figure 4. HJ4.5b isolate

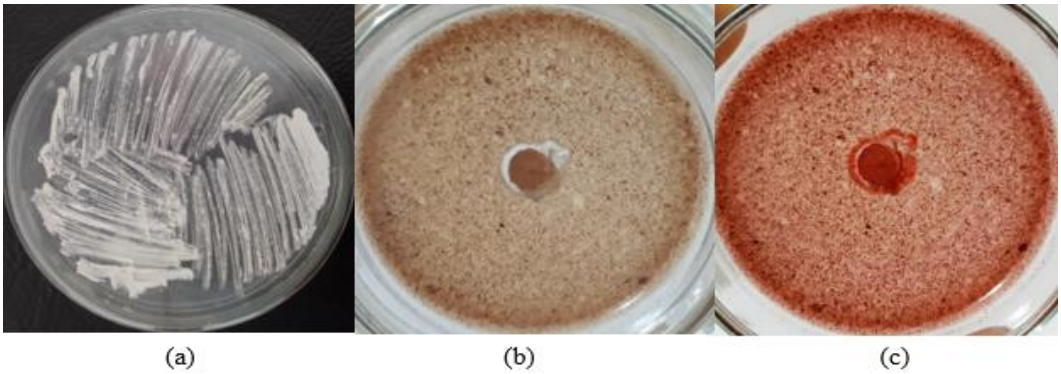

Figure 5. P2b(b).18 isolate

Caption 2 - 5:
(a) Growth of isolates on ISP4 medium on the 3rd day
(b) Growth of isolates on coffee pulp media on the 2nd day
(c) The clear zone formed after giving 0,1% congo red and rinsing with 0.2 M NaCl on the 2nd day

5. Conclusion

Actinobacteria have a high ability to produce cellulase and xylanase that can be used to hydrolyze lignocellulosic biomass of robusta coffee pulp (*Coffea canephora*). In this present study revealed 10 isolates of actinomycetes showed the ability to grow and degrade the cellulose and hemicellulose of coffee pulp on the congo red plate method. Base on the results obtained that P2b(b).3 and P2b(b).4 (xylanase activity), HJ4.5b (cellulase activity) and P2b(b).18 (cellulase and xylanase activities) are promising isolates to be continued for further research on fermentation coffee cherries.

Acknowledgment

Part of this research was funded by ABS Fund 2017 Project B02 for Anja Meryandini.

References

[1] Dilip CV, Mulaje S and Mohalkar RY 2013 *IJPSR* **4** 1730.
[2] Chandel and Silva 2013 Sustainable degradation of lignocellulosic biomass-techniques, application, and commercialization. Croatia (CU). Published by InTech
[3] Anwar Z, Gulfraz M and Irshad M 2014 *Journal of Radiation Research and Applied Sciences* **7** 163.
[4] Phitsuwan P, Sakka K and Ratanakhanokchai K 2013 *Biomass and bioenergy* **XXX** 116.
[5] Direktorat Jendral Perkebunan 2016 Statistik perkebunan Indonesia 2015-2017: Kopi. Direktorat Jendral Perkebunan Departemen Pertanian RI: Dirjen Perkebunan. Indonesia (ID). Dirjen Perkebunan Indonesia
[6] Mussatto SI, Ercilia M and Machado S 2011 *Food Bioprocess Technol*. **4** 661.
[7] Esquivel P and Jimenez VM 2011 *Food Research International* **46.**
[8] Diniyah N, Maryanto N, Ahmad S, Demi S and Achmad 2013 *J Teknologi Pertanian* **14** 73.
[9] Braham JE and Bressani R 1979 *Coffee pulp: Composition, technology, and utilization*. Ottawa. Canada (CA). International Development Research Centre.
[10] Silva CF, Vilela DM, Souza CC, Duarte WF, Dias DR. and Schwan RF 2013 *Microbiol Biotechnol*. **29** 235.
[11] Novita E, Syarief R, Noor E and Mulato S 2010 *Agrotek*. **4(1)** 76.
[12] Massawe GA and Lifa SJ 2010 *Post-Technol Innov*. **2** 41.
[13] Kurniawati N, Meryandini A and Sunarti TC 2016 *Emirates Journal of Food and Agriculture* **28(3)** 188.
[14] Gonzalez M, Coronel AR and Mancera M 2011 *Food Technol Biotechnol*. **49** 374.
[15] Saini. A Anggarwal N, Sharma A and Yadav A 2015 *Enzyme Research* **2015** 1.
[16] Boroujeni ME, Das A, Prashanthi K, Suryan S and Bhattacharya S 2012 *Journal of Biological Sciences* **12(1)** 43.
[17] Astuti E 2012 *Activity of cellulolytic and characterization actinomycetes from peat soils*. Thesis. Bogor Agricultural University, Indonesia
[18] Apriani I 2013 *Selection of actinomycetes for degradation lignocellulosic biomass*. Thesis. Bogor Agricultural University. Indonesia
[19] Shirling EB and Gottlieb D 1966 *International Journal of Systematic Bacteriology* **16(3)** 313.
[20] [AOAC] Association of Analytical Communities 1995 Official methods of analysis of the association of analytical chemists. Washington DC (US): AOAC *Int. Anal Chem*. **31** 426.
[21] Van Soest PJ, Robertson JB and Lewis BA 1991 *J Dairy Sci*. **74** 3583.
[22] Poltronieri and Rossi 2016 *Challenges* **79** 1.
[23] Goto BY and Fukunaga ET 1985 *Harvesting and processing for top quality coffee*. Univ. of Hawaii, Hawaii Inst. Trop. Agr. and Human Resources Ext. Cir. 359
[24] Tarigan EW and Towaha J 2017 *Journal of industrial and beverage crops* **4(3)** 1.
[25] Clifford MN and Wight J 1976 *J. Sci. Fd Agric*. **27** 13.
[26] Variyar PS, Ahmad R, Bhat R, Niyas Z and Sharma A 2003 *J. Agric. Food Chem*. **51** 7945.
[27] Farah A and Donangelo CM 2006 *Braz. J. Plant Physiol*. **18(1)** 23.

[28] Rachman A 1989 Pengantar Teknologi Fermentasi. Departemen Pendidikan dan Kebudayaan Direktorat Jendral Pendidikan Tinggi Pusat Antar Universitas Pangan dan Gizi Institut Pertanian Bogor. Bogor. Indonesia.
[29] Ashok P, Soccol CR, Nigam P, Brand D, Mohan R, and Roussos S 2000 *Biochem Eng*. **6** 153.
[30] Dashtban M, Schraft H and QinFungal W 2009 *Int. J. Biol. Sci*. **5(6)** 578.
[31] Johnsen and Krause 2014 *Int. J. Mol. Sci.* **15** 830.
[32] Sumardi 2005 *Isolasi, karakterisasi, dan produksi ß-mannanase ekstraseluler dari Geobacillus strearothermophilus L-07* [disertasi]. Bogor (ID): Institut Pertanian Bogor.
[33] Bui HB 2014 *Int J Recycl Org Waste Agricult*. **3(48)** 1.
[34] Payal D, Renu S and Monisha K 2014 *IJBR* **5** 438.
[35] Camila F, Sonia C and Cristiane SF 2012 *Hindawi Publishing Corporation Enzyme Research* **2012** 1.
[36] Pushpendra S, Vijay K, Bindu N and Gajrat SB 2013 *Eurasia J Biosci.* **7** 30.
[37] Lamb J and Loy T 2005 *Journal of Archaeological Science* **32** 1433.
[38] Ten LN, Im WT, Kim K, Kang MS and Lee ST 2004 *Journal of Microbiological Methods* **56** 375.
[39] Bettache A, Azzouz Z, Boucherba N, Bouche C, Hamma S, Maibeche R, Gahfif O and Benallaoua S 2018 *SAJ Biotechnology* **5** 1.
[40] Suchita N, Rup L and Kuhad RC 2006 *Current Microbiology* **53** 178.
[41] Lamia MH, Fariz Z, Sonia MA, Sevastianos R, Samia A, Veronique D and Mouloud K 2017 *Iranian J Biotech* **15** 74.
[42] Loredano P, Fabio T and Elena R 2015 *FEB Journal* **2015** 1.
[43] Federico M, Fernando V and Natalia V 2006 *Environ. Sci. Technol*. **40** 5538.