Isolation and Identification of Hemicellulolytic Bacteria from Indonesian Coffee Pulp Waste

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Abstract. Coffee pulp waste contains 25.9–33.0% hemicellulose. Hemicellulolytic bacteria possess the ability to depolymerize hemicellulose by producing hemicellulase enzymes. The objective of the research is to isolate and identify the hemicellulolytic bacteria from Indonesian coffee pulp waste. Hemicellulolytic bacteria were isolated from coffee pulp wastes of *Coffea arabica* and *C. canephora* from two coffee plantations in East Java Province, Indonesia. These isolates were selected based on the hydrolysis of xylan and xylanase activity on the xylan medium. The best isolates were identified based on 16S rDNA sequence similarity. In this research, 23 bacteria had isolated. Five isolates XRM21, XAJ25, XAJ30, XAJ31, and XAJ34 had higher hemicellulolytic activity than others. The XAJ25, XAJ31, and XAJ34 isolates had a good xylanase activity performance. The XAJ34 isolate had the highest xylanase activity (3.38 ± 0.65 U/ml). The XAJ34 isolate based on 16S rDNA sequence similarity was identified as *Bacillus aureus.* This bacterium is considered a potential indigenous isolate to degrade hemicellulose of coffee pulp waste.

Keywords: Hemicellulolytic bacteria · Coffee pulp waste · Bacillus aureus XAJ3

1 Introduction

Coffee is one of the most important Indonesian agricultural commodities produced annually. Coffee is a strategic commodity because of its ability to fulfill domestic needs and it can be exported to increase foreign exchange. According to Atlas [1] Indonesia is the fourth largest coffee producer in the world. A total of 467.8 thousand tons of coffee had been exported to five countries in the world, namely the United States, Germany, Malaysia, Italy, and Russia in 2017 (Sub Directorate of Estate Crops Statistics Indonesia, 2017) [2].

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Coffee processing could generate a lot of coffee pulp waste which contains a high level of lignocellulose compound (8.5–12.0% cellulose; 25.9–33.0% hemicellulose; and 2.03–27.4% lignin) [3]. Coffee pulp waste is also affordable and eco-friendly; therefore, it can be considered as a potential resource.

Hemicellulose is a carbohydrate. It constitutes the second biggest component after cellulose composing lignocellulose of the plant cell wall. Hemicellulose itself is made up of 100–200 different monosaccharides. It mainly consists of pentose sugars (D-xylose, D-arabinose), hexose sugars (D-mannose, D-glucose, and D-galactose), and acid sugars (glucuronide acid) [4]. Xylan is the primary hemicellulose that is found in hardwood and grass. Xylan polymers are characterized by β-1,4-linkages between xylose monomers which can be substituted with arabinose, glucuronic acid, and acetyl groups. Xylose and xylobiose repeats in xylan resemble the glucose and cellobiose repeats in cellulose [5].

Hemicellulolytic bacteria possess the ability to depolymerize hemicellulose by producing hemicellulase enzymes. The mechanism of hemicellulose degradation is actually more complex than cellulose. Hemicellulose is composed of many monomolecules; thus, hemicellulose degradation involves many enzymes compared to the cellulose degradation. Hemicellulase enzymes can break different linkages or the same linkages with different substrates. Endo-β-1,4-xylanase [EC 3.2.1.8], xylan β-1,4-xylosidase [EC 3.2.1.37], and α-L-arabinofuranosidase [EC 3.2.1.55] are the enzymes that degrade xylan hemicellulose [6, 7]. Some bacteria that possess the xylanolytic ability are *Acidothermus, Actinomadura, Alicyclobacillus, Anoxybacillus, Bacillus, Cellulomonas, Clostridium, Dictyoglomus, Enterobacter, Geobacillus, Paenibacillus, Nesterenkonia, Streptomyces, Thermoanaerobacteria*, and *Citrobacter* [8]-[10].

There has no report yet about the indigenous hemicellulolytic bacteria from Indonesian coffee pulp. The study of coffee pulp indigenous hemicellulolytic bacteria is important to understand the biodegradation agent of coffee pulp waste. This research is conducted to isolate and identify hemicellulolytic bacteria isolated from Indonesian coffee pulp waste.

2 Materials and Methods

2.1 Coffee Pulp Waste Sample Collection

The coffee pulp waste was taken from two coffee plantations in East Java Province, Indonesia. The pulp waste of *C. arabica* was collected from the Jampit coffee plantation at approximately 1349 m above sea level (8º00′46.87”S and 114º08′07.19”E). Pulp was of *C. canephora* collected from Malangsari coffee plantations located at approximately 954 m above sea level (8º21′34.34”S and 113º56′43.37”E).

2.2 Isolation of Hemicellulolytic Bacteria

Sample was 25 g of coffee pulp waste suspended into 225 ml physiological saline (0.85% NaCl solution) in a 500 ml Erlenmeyer flask as 10^{-1} dilution. The suspension was made serial dilutions of as 10^{-5}. The sample suspension as much as 100 μL at 10^{-3}, 10^{-4}, and 10–5 dilutions were spread on M9 minimal medium plate, the medium consist of

$Na_2HPO_4.12H_2O$ 15.0 g/l, KH_2PO_4 3.0 g/l, NaCl 0.5 g/l, $MgSO_4$ 0.25 g/l, NH_4Cl 1.0 g/l, bacto agar 15.0 g/l with 0.1% xylan as carbon source (m/v) [11, 12]. The experiment was carried out with two replications. Minimal medium without carbon source and Nutrient Agar medium was used as the negative and positive control respectively, to determine bacterial growth. The culture was incubated at 30°C for three days. A single colony of each bacterial isolate was purified.

2.3 Screening of Hemicellulolytic Bacteria Based on Hydrolysis of Xylan and Xylanase Activity

The screening of hemicellulolytic bacteria based on hydrolysis of xylan was carried out with three replications according to Kasana [13], Meddeb-Movelhi [14], and Kanimozhi [15]. The one loop of hemicellulolytic bacteria was cultured into 10.0 ml Nutrient Broth medium in shaking incubators with 120 rpm agitation at room temperature overnight. Cell density of each culture of the bacteria was adjusted with 1.4 optical density (OD). Each culture suspension of the bacteria isolate (40 μl) was inoculated into the well with 0.5 cm diameter on M9 solid medium containing1.0% xylan. Bacterial cultures were incubated at 30 °C for three days. After incubation, the bacterial medium was flooded with 0,1% iodine solution for five minutes. The clear zone around the wells were measured using digital calipers. The data of clear zones that indicate xylanase activity was an analysis of variance continued by the Duncan test with 95% significance difference using SPSS 16. The bacteria isolate with high enzyme activity were assayed based on xylanase activity quantitatively.

The potential of hemicellulolytic bacteria with xylanase activity was assayed with three replications according to Chakdar [5] and Sheng [16]. The isolates of hemicellulolytic bacteria were cultured into 10.0 ml of NB medium in a shaking incubator with 120 rpm agitation at room temperature overnight. Cell density of the culture was adjusted with 1.4 OD. Ten percent of the culture (v/v) was inoculated into an M9 liquid medium containing 1.0% yeast extract and 1.0% xylan. The culture was incubated in shaking incubators with 120 rpm agitation at room temperature for three days. The bacterial culture (1.5 ml) was centrifuged at 10.000 rpm, 30 °C for 10 min. The supernatant contained crude enzymes of hemicellulase. Xylanase activity was measured using Nelson and Somogyi method [17, 18]. One unit (U) of xylanase activity is defined as the number of enzymes needed to produce one milliliter of reducing sugars (xilose) per minute at 37 ºC.

2.4 Identification and Biochemical Characterization of Potential Hemicellulolytic Bacteria

The potential bacteria were identified based on 16S rDNA sequence and it was characterized by their phenotype. The culture of bacterial isolates on Nutrient Agar (NA) medium at 24 h old was characterized by the cultural morphology. The bacterial cell morphology was observed using Olympus CX21 with 1000 magnification. Biochemical characteristics of bacteria culture were analyzed using Microbact (Oxoid) 24E (12A (12E) + 12B) kit. Potential bacteria isolates were grown on a blood agar medium to assay pathogenicity.

The chromosomal DNA of potential bacteria was isolated using CTAB method [19] [20]. The sequence of 16S rDNA was amplified using 27f primer (5'- GAGAGTTTGATCC-TGGCTCAG-3') and 1495r primer (5'-CTACGGCTACCTTGTTACGA-3') [21]. The phylogeny tree was constructed using Neighbor-Joining method and Jukes-Cantor algorithm with 5.000 times bootstrap with MEGA 6 programme.

3 Results and Discussion

3.1 The Potency of Hemicellulolytic Bacteria

Hemicellulolytic bacteria were able to degrade hemicellulose. Hemicellulose is the second dominant component composing lignocellulose of the plant cell wall.

Xylan is a common and primary hemicellulose found in hardwood and grass that can be used to help identify hemicellulolytic bacteria [4, 10]. Based on growth ability on M9 minimal medium contain 0.1% xylan, this research found 23 isolates as hemicellulolytic bacteria which consist of 16 isolates which were obtained from robusta coffee pulp waste and 7 isolates were obtained from arabica coffee pulp waste (Table 1). These isolates were able to grow with xylan as the sole carbon source.

Hemicellulolytic bacteria were selected based on their ability to degrade xylan that indicated by clear zones around bacteria colonies on xylan medium colored with iodine [22]-[25]. The results showed that those hemicellulolytic bacteria isolates had different xylan degradation activities. Isolate XAJ30 has highest xylanolytic activity with diameter of clear zones 4.2 ± 0.15 cm, followed by XRM21 (4.0 ± 0.13 cm), XAJ25 (3.0 ± 0.23 cm), XAJ31 (2.5 ± 0.06 cm), and XAJ34 (2.5 ± 0.01 cm) (Table 1). This result was similar with Eida [26] who found seven hemicellulolytic bacterial isolates from coffee pulp compost, were CRCB2, CRCB3, CRCB4, CRCB5, CRCB6, CRCB7, CRCB8 with clear zone diameter on xylan medium: 9.8 ± 2.9; 11.3 ± 1.7; 8.4 ± 1.0; 7.7 ± 0.6; 15.9 ± 2.2; 9.9 ± 0.6; and 14.7 ± 0.8 cm respectively. They identified CRCB1 as *Streptomyces*, CRCB9 and CRCB10 as *Cohnella* with xylanolitic activity 5.8 ± 1.1cm, 14.4 ± 0.6 cm, and 17.3 ± 3.3 cm respectively.

Xylanase activity was used as a parameter to analyze endoxylanase activity. These results showed that isolated XAJ34 performed the xylanase activity (39.30 ± 2.08 U/ml) within 72 h incubation on medium with 1.0% xylan as sole carbon source (Fig. 1). The xylanase activity of XAJ34 was the same as XAJ31 and XAJ25 with enzyme activity 31.48 ± 10.84 U/ml and 31.07 ± 11.78 U/ml respectively. This result showed that these isolates had an important role on coffee pulp waste degradation.

Xylanase activity of XAJ34 was lower compared to *Bacillus subtilis* EFB-11 (42.33 U/ml) incubated for 72 h [27], but it was higher than GSM-30 (37.33 U/ml) incubated for 48 h [28]. The differences of xylanase activity were influenced by genetic characteristics of particular species or strains. These differences were also affected by substrates used to grow the bacteria. Similarly, Sanghi [29] found that different substrates affected the activity of the xylanase enzyme of *Bacillus subtilis* ASH. Xylanase enzyme activity was stimulated by the addition of yeast extract, peptone, and meat extract, but inhibited by the addition of glucose. *Bacillus pumilus* CS1 was isolated from a withered corn cob performing high xylanase activity (132.0 ± 0.09 U/ml) at 55 °C and pH 8.0 [30]. Attri

Table 1. The hemicellulose activity of bacteria isolated from coffee pulp waste

No	Pulp waste	Isolate	Diameter of enzyme activity (cm)
1	*C. canephora*	XRM2	2.1 ± 0.17^{ef}
2	*C. canephora*	XRM3	0.4 ± 0.00^{a}
3	*C. canephora*	XRM4	1.2 ± 0.04^{c}
4	*C. canephora*	XRM5	2.4 ± 0.12^{h}
5	*C. canephora*	XRM6	1.3 ± 0.20^{c}
6	*C. canephora*	XRM7	1.6 ± 0.34^{de}
7	*C. canephora*	XRM10	2.1 ± 0.06^{e}
8	*C. canephora*	XRM13	2.0 ± 0.04^{e}
9	*C. canephora*	XRM14	0.7 ± 0.02^{b}
10	*C. canephora*	XRM17	2.0 ± 0.04^{e}
11	*C. canephora*	XRM18	0.4 ± 0.00^{a}
12	*C. canephora*	**XRM21**	**$4.0 \pm 0{,}03^{i}$**
13	*C. canephora*	XRM35	0.4 ± 0.00^{a}
14	*C. canephora*	XRM37	2.3 ± 0.01^{fgh}
15	*C. canephora*	XRM38	2.1 ± 0.10^{ef}
16	*C. canephora*	XRM40	1.4 ± 0.23^{cd}
17	*C. arabica*	**XAJ25**	**3.0 ± 0.23^{i}**
18	*C. arabica*	**XAJ30**	**4.2 ± 0.15^{k}**
19	*C. arabica*	**XAJ31**	**2.5 ± 0.06^{h}**
20	*C. arabica*	XAJ32	0.4 ± 0.00^{a}
21	*C. arabica*	XAJ33	2.2 ± 0.11^{efg}
22	*C. arabica*	**XAJ34**	**2.5 ± 0.01^{h}**
23	*C. arabica*	XAJ36	0.6 ± 0.08^{ab}

and Garg [29] found some bacterial isolates are able to produce xylanase, cellulase, and pectinase from land for dumping fruits, vegetables, wood materials, and dairy factories. Bacterial isolates of GSM-30 had highest activity of xylanase, cellulase, and pectinase was 37.33 U/ml, 0.6 U/ml, and 2.11 U/ml respectively.

3.2 Taxonomy and Biochemical Characteristics of Hemicellulolytic Bacteria XAJ34

Based on the phenotype characterization, culture of XAJ34 showed creamy, irregular shape, elevated umbonate and slimy textures. The bacteria cell was rod-shaped 1.3 to 1.7 μm length and 0.6 to 0.8 μm diameter (Fig. 2).

Based on the biochemical test, *B. aureus* XAJ34 showed phenotype characteristics were aerobic and positive-Gram which able to ferment mannitol and sucrose but it

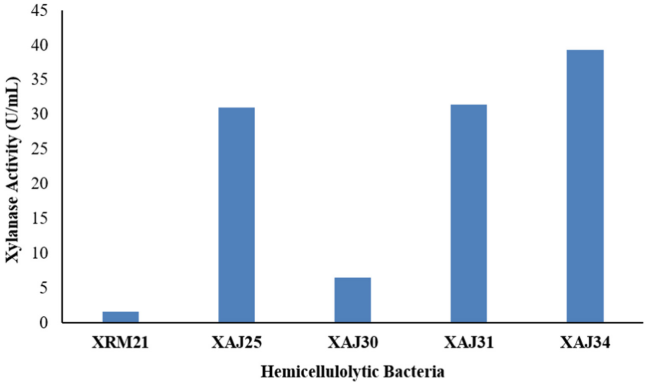

Fig. 1. Xylanase activity of hemicellulolytic bacteria isolated from coffee pulp waste.

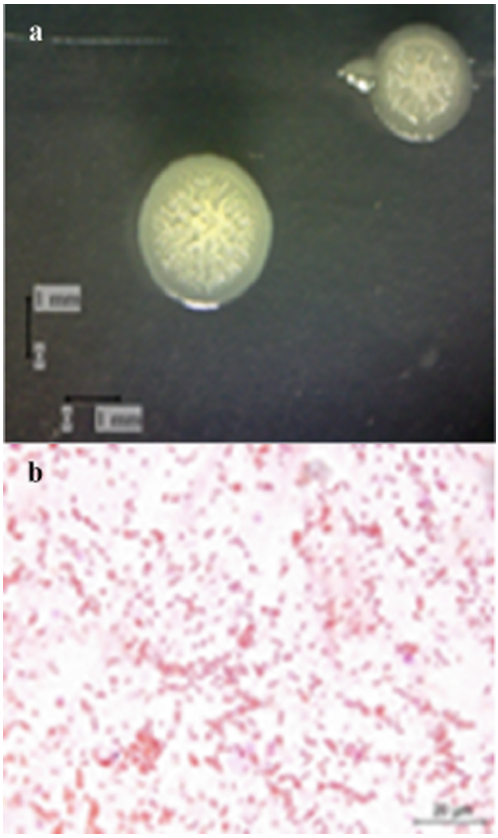

Fig. 2. Morphology of colony and cell of *Bacillus aureus* XAJ34 on Nutrient Agar (NA) medium at 24 h (a. morphology of colony b. morphology of cell).

Table 2. Biochemical characteristic of *Bacillus aureus* XAJ34

Characteristics	Reaction	Characteristics	Reaction
Gram-positive	+	Production of indole pyruvate by deamination of tryptophan	–
Catalase	+	Gelatin liquefaction	–
Lysine decarboxylase	–	Malonate inhibition	–
Ornithine decarboxylase	–	Inositol fermentation	–
H_2S production	–	Sorbitol fermentation	–
Glucose fermentation	–	Rhamnose fermentation	–
Mannitol fermentation	+	Sucrose fermentation	+
Xylose fermentation			
Hydrolysis of o-nitrophenyl-ß-d-galactopyranoside (ONPG) by action of ß-galactosidase	–	Arabinose fermentation	–
Indole production from tryptophan	–	Adonitol fermentation	–
Urea hydrolysis	–	Raffinose fermentation	–
Acetoin production (Voges-Proskaüer reaction)	–	Salicin fermentation	–
Citrate utilization (citrate is the only source of carbon)	–	Arginine dehydrolase	–

was unable to ferment other carbohydrates such as glucose, inositol, sorbitol, lactose, adonitol, raffinose, xylose, rhamnose, arabinose, and salicin. This isolate was not possessed enzymes such as lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, and tryptophan deaminase nor produce acetoin, indole, H_2S, and Na malonate. In addition, this isolate was unable to hydrolyze ONPG, urea, and gelatin (Table 2).

Based on phylogenetic identification, isolate XAJ34 had 99.9% similarity of 16S rDNA sequence with *Bacillus aureus* 24K (Fig. 3). The *B. aureus* 24K was isolated from the air at altitude 24, 28, and 41 km from the ground level. The codes given to the bacterium were MTCC 7303T and JCM 13348T [30]. *B. subtilis* [27, 29], *B. amyloliquefaciens* [17, 31], and *Thermobacillus xylanilyticus* [32] are the examples of species from Genera of *Bacillus* that had xylanase activity. Other genera that possess hemicellulolytic activity are *Pedobacter* and *Mucilaginibacter* [33].

B. aureus XAJ34 growth curve on M9 medium contains 0.1% Yeast Extract and 0.1% xylan showed logarithmic phase until 24 h of incubation with cell density 2.46 x 108 cell/ml and generation time 6.94 h. After that, the isolate encountered the stationary phase at 42 h (Fig. 4). Based on the growth curve, it is recommended that 24 h incubation is the most appropriate time for recovery of XAJ34 isolate as the starter for fermenting coffee pulp.

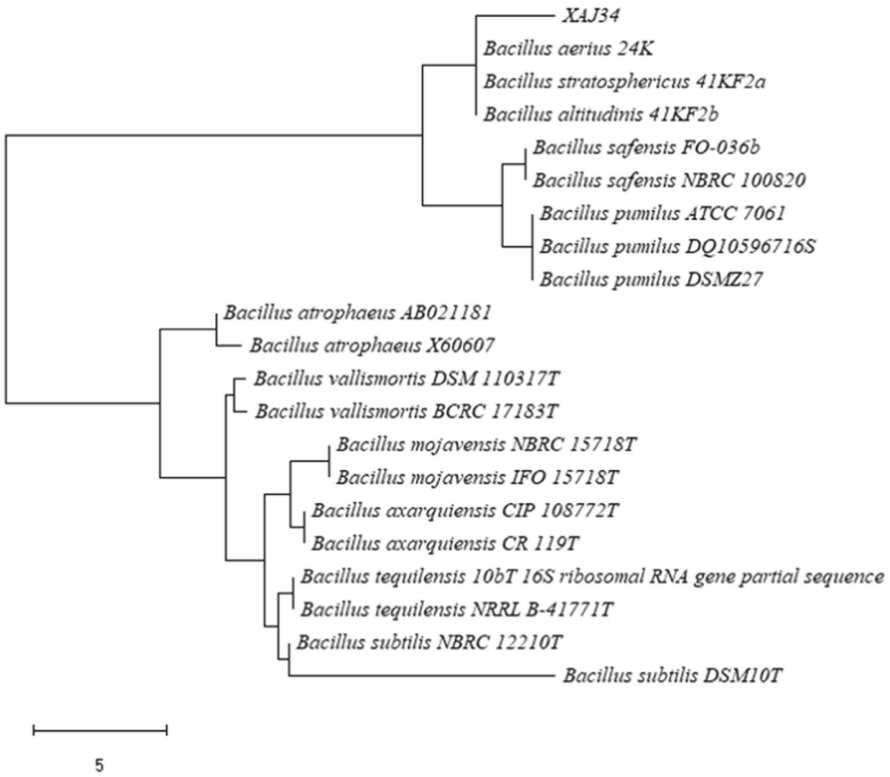

Fig. 3. Phylogeny tree of *Bacillus aureus* XAJ34 and reference isolates based on 16S rDNA sequence similarity with neighbor-joining algorithm.

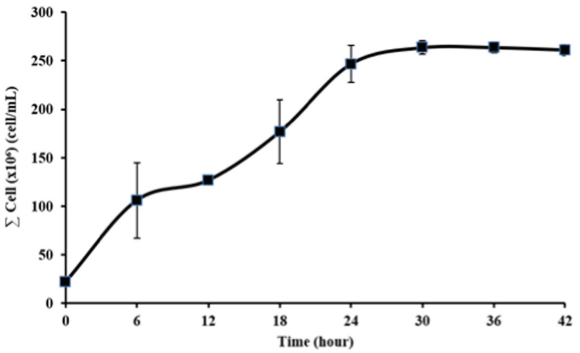

Fig. 4. The growth curve of *Bacillus aureus* XAJ34 on M9 medium contains 0.1% yeast extract and 0.1% xylan on 24 h of incubation.

The hemicellulolytic bacteria XAJ34 isolate has been successfully isolated from coffee pulp waste. These bacteria are considered as a potential indigenous isolate to degrade hemicellulose of coffee pulp waste.

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References

1. W. Atlas, "Top coffee producing countries wordatlas," 2017. http://www.worldatlas.com/articles/topcoffee-producing-countries.html. (accessed Aug. 30, 2018).
2. Sub Directorate of Estate Crops Statistics Indonesia, "Indonesian Coffee Statistics 2017," 2017.
3. S. Arimurti, "The potency of lignocellulolytic and caffeine-degrading bacteria from East Java coffee plantation as a coffee pulp decomposer," Brawijaya University, 2018.
4. H. Abdellaoui, R. Bouhfid, and A. E. K. Qaiss, "Lignocellulosic Fibres Reinforced Thermoset Composites: Preparation, Characterization, Mechanical and Rheological Properties," 2018, pp. 215–270. https://doi.org/10.1007/978-3-319-68696-7_5.
5. H. Chakdar *et al.*, "Bacterial xylanases: biology to biotechnology," *3 Biotech*, vol. 6, no. 2. Springer Verlag, 2016. doi: https://doi.org/10.1007/s13205-016-0457-z.
6. E. Ben Guerrero *et al.*, "Prospection and evaluation of (HEMI) cellulolytic enzymes using untreated and pretreated biomasses in two Argentinean native termites," *PLoS One*, vol. 10, no. 8, Aug. 2015, https://doi.org/10.1371/journal.pone.0136573.
7. V. M. Vasconcellos, P. W. Tardioli, R. L. C. Giordano, and C. S. Farinas, "Addition of metal ions to a (hemi)cellulolytic enzymatic cocktail produced in-house improves its activity, thermostability, and efficiency in the saccharification of pretreated sugarcane bagasse," *N. Biotechnol.*, vol. 33, no. 3, pp. 331–337, 2016, doi: https://doi.org/10.1016/j.nbt.2015.12.002.
8. M. Maki, K. T. Leung, and W. Qin, "The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass," 2009. [Online]. Available: http://www.biolsci.org500
9. A. Bhalla, N. Bansal, S. Kumar, K. M. Bischoff, and R. K. Sani, "Improved lignocellulose conversion to biofuels with thermophilic bacteria and thermostable enzymes," *Bioresource Technology*, vol. 128. Elsevier Ltd, pp. 751–759, 2013. https://doi.org/10.1016/j.biortech.2012.10.145.
10. S. L. A. Sari *et al.*, "Cellulolytic and hemicellulolytic bacteria from the gut of Oryctes rhinoceros larvae," *Biodiversitas*, vol. 17, no. 1, pp. 78–83, Apr. 2016, https://doi.org/10.13057/biodiv/d170111.
11. H. L. Woo, T. C. Hazen, B. A. Simmons, and K. M. DeAngelis, "Enzyme activities of aerobic lignocellulolytic bacteria isolated from wet tropical forest soils," *Syst. Appl. Microbiol.*, vol. 37, no. 1, pp. 60–67, 2014, https://doi.org/10.1016/j.syapm.2013.10.001.

12. M. Vellaichamy and I. Fiii, "Growth kinetics of Bacillus subtilis in lignocellulosic carbon sources Popularisation of ICAR-CIRCOT technologies on bio-enriched compost production and oyster mushroom cultivation using cotton stalks among the cotton growing farmers of Vidarbha region View project Nutrigenomics View project International Journal of Microbiology Research," vol. 6, no. 2, pp. 570–574, 2014, [Online]. Available: http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234
13. R. C. Kasana, R. Salwan, H. Dhar, S. Dutt, and A. Gulati, "A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine," *Curr. Microbiol.*, vol. 57, no. 5, pp. 503–507, 2008, doi: https://doi.org/10.1007/s00284-008-9276-8.
14. F. Meddeb-Mouelhi, J. K. Moisan, and M. Beauregard, "A comparison of plate assay methods for detecting extracellular cellulase and xylanase activity," *Enzyme Microb. Technol.*, vol. 66, pp. 16–19, 2014, doi: https://doi.org/10.1016/j.enzmictec.2014.07.004.
15. P. . Kanimozhi, K and Nagalakshmi, "Xylanase production from Aspergillus niger by solid state fermentation using agricultural waste as substrate," *Int. J. Curr. Microbiol. Appl. Sci.*, vol. 3, no. 3, pp. 437–446, 2014.
16. P. Sheng, J. Xu, G. Saccone, K. Li, and H. Zhang, "Discovery and characterization of endo-xylanase and β-xylosidase from a highly xylanolytic bacterium in the hindgut of Holotrichia parallela larvae," *J. Mol. Catal. B Enzym.*, vol. 105, pp. 33–40, 2014, doi: https://doi.org/10.1016/j.molcatb.2014.03.019.
17. S. Singh, V. S. Moholkar, and A. Goyal, " Isolation, Identification, and Characterization of a Cellulolytic Bacillus amyloliquefaciens Strain SS35 from Rhinoceros Dung ," *ISRN Microbiol.*, vol. 2013, pp. 1–7, 2013, doi: https://doi.org/10.1155/2013/728134.
18. M. M. Rashad, A. E. Mahmoud, M. U. Nooman, H. A. Mahmoud, A. E. D. M. M. El-Torky, and A. T. Keshta, "Production of antioxidant xylooligosaccharides from lignocellulosic materials using Bacillus amyloliquifaciens NRRL B-14393 xylanase," *J. Appl. Pharm. Sci.*, vol. 6, no. 6, pp. 30–36, 2016, https://doi.org/10.7324/JAPS.2016.60606.
19. K. Wilson, "Preparation of Genomic DNA from Bacteria.," in *In: Current Protocols in Molecular Biology 5th edn.*, New York, 2003.
20. S. Arimurti *et al.*, "Screening and identification of indigenous cellulolytic bacteria from Indonesian coffee pulp and investigation of its caffeine tolerance ability," vol. 13, no. pp. 109–116, 2017.
21. S. Arimurti, T. Ardyati, Y. Nurani, T. A. Siswoyo, and S. Suharjono, "Degradation of caffeine by Pseudomonas monteilii KRM9," *Malays. J. Microbiol.*, vol. 14, no. 1, pp. 55–60, 2018, https://doi.org/10.21161/mjm.110217.
22. X. Li and H. Y. Yu, "Purification and characterization of an organic-solvent-tolerant cellulase from a halotolerant isolate, Bacillus sp. L1," *J. Ind. Microbiol. Biotechnol.*, vol. 39, no. 8, pp. 1117–1124, Aug. 2012, doi: https://doi.org/10.1007/s10295-012-1120-2.
23. D. J. Jiménez, E. Korenblum, and J. D. Van Elsas, "Novel multispecies microbial consortia involved in lignocellulose and 5-hydroxymethylfurfural bioconversion," *Appl. Microbiol. Biotechnol.*, vol. 98, no. 6, pp. 2789–2803, 2014, doi: https://doi.org/10.1007/s00253-013-5253-7.
24. V. Gowdaman *et al.*, "Bioprospecting of Cellulase Producing Extremophilic Bacterial Isolates from India Related papers Marine ext remophiles: a source of hydrolases for biot echnological applicat ions Gabriel Dalmaso Dehalogenase producing bact eria from ext reme environment : A review Mohamed Edbeib T he Import ance of T hermophilic Bact eria in Biot echnology Bioprospecting of Cellulase Producing Extremophilic Bacterial Isolates from India," 2014. [Online]. Available: www.sciencedomain.org
25. and T. P. Riddech, N., N. Saharm, C. Chaisawang, P. Pongtongmee, S. Boonchern, P. Sarin, "Multifunctional cellulolytic activities from Streptomyces osmaniensis for agricultural and enzyme industry," *Malays. J. Microbiol.*, pp. 1823–8262, 2015.

26. M. F. Eida, T. Nagaoka, J. Wasaki, and K. Kouno, "Isolation and characterization of cellulose-decomposing bacteria inhabiting sawdust and coffee residue composts," *Microbes Environ.*, vol. 27, no. 3, pp. 226–233, 2012, https://doi.org/10.1264/jsme2.ME11299.
27. M. H. Nawawi, R. Mohamad, P. M. Tahir, and W. Z. Saad, " Extracellular Xylanopectinolytic Enzymes by Bacillus subtilis ADI1 from EFB's Compost ," *Int. Sch. Res. Not.*, vol. 2017, pp. 1–7, Apr. 2017, doi: https://doi.org/10.1155/2017/7831954.
28. G. G. Sampan Attri, "Isolation of microorganisms simultaneously producing xylanase, pectinase and cellulase enzymes using cost effective substrates," 2014. www.jibresearch.com.
29. A. Sanghi, N. Garg, J. Sharma, K. Kuhar, R. C. Kuhad, and V. K. Gupta, "Optimization of xylanase production using inexpensive agro-residues by alkalophilic Bacillus subtilis ASH in solid-state fermentation," *World J. Microbiol. Biotechnol.*, vol. 24, no. 5, pp. 633–640, May 2008, doi: https://doi.org/10.1007/s11274-007-9521-5.
30. V. A. Mahilrajan and S., S. Balakumar, "Screening and identification of a thermophilic and alkalophilic bacterium producing xylanase," *Adv. Appl. Sci. Res.*, vol. 3, no. 1, pp. 242–250, 2012.
31. S. Shivaji *et al.*, "Bacillus aerius sp. nov., Bacillus aerophilus sp. nov., Bacillus stratosphericus sp. nov. and Bacillus altitudinis sp. nov., isolated from cryogenic tubes used for collecting air samples from high altitudes," *Int. J. Syst. Evol. Microbiol.*, vol. 56, no. 7, pp. 1465–1473, Jul. 2006, doi: https://doi.org/10.1099/ijs.0.64029-0.
32. H. Rakotoarivonina, B. Hermant, N. Monthe, and C. Rémond, "The hemicellulolytic enzyme arsenal of Thermobacillus xylanilyticus depends on the composition of biomass used for growth," *Microb. Cell Fact.*, vol. 11, Dec. 2012, doi: https://doi.org/10.1186/1475-2859-11-159.
33. R. López-Mondéjar, D. Zühlke, D. Becher, K. Riedel, and P. Baldrian, "Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems," *Sci. Rep.*, vol. 6, 2016, doi: https://doi.org/10.1038/srep25279.