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Isolation and Characterization of Cellulose and Cyanide Degrading Bacteria from Cassava Waste as Inoculants in Feed Fermentation

Hera Dwi Triani[1],Yetti Marlida[2*], Ahadiyah Yuniza[2], Husmaini[2] and Wulansih Dwi Astuti[3]

[1]Doctoral Student Animal Feed Science, Faculty of Animal Husbandry, Andalas University, Padang, Indonesia 25163
[2]Departement of Feed and Nutrition , Faculty of Animal Husbandry, Andalas University, Padang, Indonesia, 25163
[3]Researcher of Research Center for Applied Zoology, National Reseearch and Inovation Agency (BRIN) Cibinong, Bogor, Indonesia, 16911
***Corresponding author:** yettimarlida@ansci.unand.ac.id

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ABSTRACT

Cassava waste has the potential to be used as duck feed; however, there are limiting factors such as high crude fiber content (27.15%) and cyanide levels (300-500ppm). Therefore, feed processing technology is required, utilizing fermentation with cellulolytic and cyanolytic bacterial inoculants capable of degrading cellulose and cyanide. The isolation of bacteria was conducted from cassava waste, including leaves and skins, using the pour-plate method on selective media containing carboxymethyl cellulose (CMC) for cellulose degradation and potassium cyanide (KCN) for cyanide degradation. The selected bacteria showing clear zone activity on their respective selective media were further tested for cellulase and β-glucosidase enzyme activity. Subsequently, morphological and biochemical tests were performed. The research results revealed that four isolates exhibited the ability to degrade cellulose and cyanide. These isolates were identified as HA1, HB2, HT3, and HT4, based on the clear zones they produced, which were converted into cellulolytic and cyanolytic indices. HA1 showed the highest degradation capability, with a cellulolytic index of HA1=2.08, HB2=1.89, HT3=1.75, and HT4=0.81, and a cyanolytic index of HA1=1.03, HB2=0.67, HT3=0.43, and HT4=0.81. Cellulase activity for each isolate was as follows: HA1=7.58U/mL, HB2=1.89U/mL, HT3=1.75U/mL, and HT4=0.81 U/mL, while β-glucosidase activity was: HA1=0.78U/mL, HB2=0.95U/mL, HT3=0.81U/mL, and HT4=1.00U/mL. Biochemical and morphological tests confirmed that all four isolates were rod-shaped, gram-positive bacteria (bacilli) with distinct strains for each. The strains of bacteria were Bascillus sp1, Bacillus sp2, Bacillus sp3 and Bacillus sp4.

Key words: Cellulolytic, Cyanolytic, Cellulase, β-glucosidase, Cassava Waste.

INTRODUCTION

West Sumatra, Indonesia, boasts a variety of cassava-based processed foods that are local products. Consequently, there is a significant amount of cassava waste with the potential to be used as duck feed. According to statistical data from Central Statistics Agency (2021), cassava production in West Sumatra reached 153,412.02tons, with peels accounting for 5-15% of the cassava production and leaves comprising 13% with the ratio of root: leaves is 20:3 (Umami 2019). Cassava leaves, which can be utilized as feed, yield around 10 tons per hectare and are characterized by a high crude protein content of 33.8–37.4%, Metabolisme Energy of 1800kcal/kg (Morgan and Mingan 2016), crude fiber content of 20.24% (Hernawan et al. 2016) and beta-carotene levels ranging from 298.00 to 816.92µg/g (Sumiati et al. 2020). In contrast, cassava peels contain 27.17% crude fiber (Mirzah and Muis 2015) and 6.78% crude protein (Oloruntola 2018). Cassava waste in the form of peels contains 6.78% crude protein, 27.18% crude fiber, and 1310% metabolic energy (Triani and Mahyudin 2014). cassava peel is low in protein and high in crude fiber and with a cyanide content of 650 and 310mg/kg depending on the variety and bitter taste, the protein content of peel meal is approximately 46 to 55g/kg (Morgan and Mingan 2016). The resulting cassava peel waste currently poses a disposal problem but has the potential to become an important resource such as feed ingredients if exploited properly by biotechnological systems (Obadina et al. 2006).

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The primary challenge, which acts as a limiting factor in utilizing cassava waste as feed, lies in the presence of anti-nutrients, specifically cyanide, which can prove fatal to livestock. Cyanide exerts its negative effects by interfering with cellular respiration through its binding to the cytochrome oxidase enzyme, thereby impeding tissue oxygen utilization (Suharti et al. 2021). Cassava leaves, in particular, contain toxic levels of cyanide, as documented by Anjani et al. (2021), with cyanide concentrations in cassava leaves ranging from 200-300ppm. Furthermore, the elevated levels of crude fiber, measuring 27.17% in the peels and 20% in cassava leaves, surpass the tolerance threshold for poultry, which can only accommodate a maximum of 7% crude fiber according to the Indonesian Standar National (2016) standards of 2016. Similarly, the accepted cyanide content in poultry diets is limited to 50ppm.

Conventional methods such as drying, heating, and boiling have been employed to reduce the cyanide content in cassava peels and leaves. However, these methods have proven to be less effective and also cause the loss of some of the nutrients in the cassava leaves, this is because damage to some of the leaf and skin cells only releases some of the linamarase so only some of the cyanogenic compounds are converted into HCN and some of the cyanogen remains in the cassava leaves (Ngudi et al. 2003), therefore The fermentation method is the right method for processing cassava waste. therefore The fermentation method is the right method for processing cassava waste. Fermentation technology, as recommended by Oloruntola (2018), has emerged as a preferred approach for processing cassava and its waste to reduce crude fiber. This is primarily due to its recognized safety, as it does not leave residues in poultry products. Moreover, fermentation can enhance the palatability of feed ingredients, leading to increased feed consumption and subsequently boosting poultry productivity (Oloruntola, 2018).

Fermentation biotechnology is the preferred method for processing cassava leaves and peels. This method not only reduces cyanide content but also increases protein levels while decreasing crude fiber (Adeleke et al. 2017). In contrast, cooking methods can lead to a loss of up to 58% of protein content (Hawashi et al. 2019). Previous research on the fermentation of cassava waste has typically focused on employing microbes that target specific substrates, such as crude fiber or cyanide, individually. Consequently, the utilization of fermented cassava waste in poultry or duck rations has not been optimal. The application of fermentation technology using cellulolytic microbes has proven effective in reducing crude fiber (Oloruntola, 2018). For instance, the fermentation of cassava waste using *Leuconostoc mesenteroides* bacteria resulted in a remarkable 90.43% reduction in cyanide content but did not significantly reduce crude fiber (Sandi 2010). Similarly, fermentation using *Saccharomyces cerevisiae* led to a 37.57% reduction in crude fiber but did not significantly reduce cyanide acid levels (Triani and Mahyudin 2014). Moreover, the use of *Bacillus amyloliquefaciens* bacteria as an inoculant in the fermentation of cassava waste increased protein content by 45.34% but only achieved a modest 13.48% reduction in crude fiber (Mirzah and Muis 2015).

Fermentation technology applied to cassava waste, utilizing cellulose-degrading (cellulolytic) and cyanide-degrading (cyanolytic) bacteria as inoculants isolated from cassava waste itself, presents a more effective approach for the degradation of cellulose and cyanide in feed. This approach is particularly well-suited to the local environment where cassava is cultivated. Since the bacteria originate from the same ecosystem, the use of fermented cassava waste in duck feed is expected to be highly efficient and environmentally appropriate.

MATERIALS AND METHODS

Ethical Approval

This research did not require ethical approval because we did not use animals but instead used isolates of cellulose and cyanide degrading bacteria that were isolated from cassava waste.

Isolation of Cellulose Degrading Bacteria (Cellulolytic)

The samples come from cassava waste which is peeling and leave cassava fermented for 7 days, 10g of the sample was dissolved in 100mL of physiological solution and diluted to 10^{-6}, then 0.5mL of solution at dilutions of 10^{-4}, 10^{-5} and 10^{-6} added to a Petri dish containing nutrient agar (NA), incubated for 24h at 37˚C. Bacteria that grew in Petri dishes were placed on a slanted medium and incubated for 24h at 37˚C. Single bacteria on slanted media were grown on cellulase selective media containing CMC, the selective media consisted of (for 100mL): 0.02g $MgSO_4$, 0.075g KNO_3, 1g CMC, 0.05g K_2HPO_4, 0.02g $FeSO_4$, 0.04g $CaCl_2$, 0.2g yeast, 1.8g agar and 0.1g glucose. After incubation for 24h at 37˚C, drops of congo red (0.1g congo red+50mL of ethanol), after 15min, washed with physiological NaCl and measured the clear zone diameter and the diameter of the isolated colony, then calculated the cellulolytic index with the formula:

$$Cellulolytic\ index = \frac{clear\ zone\ diameter - clear\ zone\ colonies}{clear\ zone\ colonies}$$

Isolation of Cyanide-Degrading Bacteria

The isolates that displayed clear zones on cellulose-were further cultured on cyanide-selective media containing 0.25g/L KCN in Petri dishes, they were incubated for 48h at 37°C, and the clear zone and colony zone were measured. The cyanide-selective medium composition for 100mL included 0.02g $MgSO_4$, 0.075g KNO_3, 0.025g KCN, 0.05g K_2HPO_4, 0.02g $FeSO_4$, 0.04g $CaCl_2$, 0.2g yeast, 1.8g agar, and 0.1g glucose.

Cellulase Enzyme Activity

The cellulase enzyme activity test was carried out using the Nelson method. Briefly, 1mL crude enzyme + 1mL extract (0.5 CMC + 10mL buffer) was incubated at 40°C for 30min in a water bath shaker. Then 1mL of crude enzyme which has been mixed with buffer extract and CMC plus 1mL of Nelson AB solution was heated in boiling water for 20min, allowed to cool, then added 1mL of phosphomolybdate + 7mL of distilled water, reading was taken in a spectrophotometer at a wavelength of 575nm and a standard curve was drawn.

$$\text{Cellulase activity (U/mL)} = \frac{X \times P \times 1000}{t \times BM}$$

X = standard curve conversion result
P = Dilution
T = incubation time
BM = Molecular Weight.

β-Glucosidase Enzyme Activity

The substrate is p-NPG at a concentration of 0.1% (w/v). Enzyme extract, citrate buffer solution with a pH of 5.0, and the substrate were preincubated for 10min at 50°C. Subsequently, 0.5mL of the enzyme extract with the appropriate dilution was mixed with 0.5mL of citrate buffer and 0.5mL of the substrate. This solution was then incubated at 50°C for 60min. Then, 1.0mL of 1M Na_2CO_3 solution was added, followed by vortexing, and the absorbance was measured using a spectrophotometer at a wavelength of 400nm. For the control, the same composition was prepared, but the enzyme was added after the inclusion of 1.0mL of 1M Na_2CO_3 solution. A blank solution was created using 1mL of distilled water, 0.5mL of buffer solution, and 1mL of Na_2CO_3. Standard solutions were prepared using a p-nitrophenol solution, with concentrations from 0 to 30µg/mL.

$$\text{β-glucosidase activity (U/mL)} = \frac{\text{(nitropenal sample – control) x dilution factor}}{\text{incubation time x BM nitropenol}}$$

Characterization of Cellulose and Cyanide-Degrading Isolates

H2S and Gas Production Test

The bacterial isolate was inoculated onto Triple Iron Sugar Agar (TSIA) medium using an inoculation needle, followed by incubation for 24-48h. A positive test for H2S production was indicated by the development of a black color within the medium. and the presence of gas accumulating at the bottom.

SIM Test

A single dose of the bacterial isolate was inoculated into 3mL of SIM (Sulfide, Indole, Motility) medium within a test tube. The culture was then incubated for 24h at 37°C. To perform the indole test, Kovacs reagent was introduced. A positive test result was identified by the appearance of a red color. For the motility test, a positive result was observed when the medium became cloudy.,

Methyl Red (MR) and Voges-Proskauer (VP) Tests

Bacterial cultures obtained from CMC media were inoculated onto MR/VP media and incubated for 24h at 37°C. The bacterial growth in the MR/VP culture was then divided into two separate tubes. For the MR test, 2-3 drops of methyl red reagent were added, and for the VP test, 2-3 drops of 5% alpha-naphthol reagent were introduced.

Citrate Test

Bacterial colonies were inoculated onto citrate media, followed by incubation at 37°C for 24h. Bacteria that utilize citrate as a carbon source produce sodium carbonate, which is alkaline. Consequently, the presence of a bromothymol blue indicator results in a blue coloration of the media.

Carbohydrate Fermentation Test

Bacterial isolates were taken in a single dose and introduced into a carbohydrate medium containing glucose, sucrose, lactose, and mannitol, along with bromthymol blue (BTB) and phenol red as pH indicators. The medium, with the bacterial isolate, was then incubated for 24h. A change in color to red indicates the absence of acid production, whereas a yellow color indicates the presence of acid.

Urea Test

Bacterial isolates were inoculated onto urea agar (slanted agar) and incubated at 37°C for 48h. The observation focused on any color changes that occurred. A red-purple color within the media indicated a positive reaction by the bacteria.

RESULTS

The research results were found of four isolates, namely HA1, HB2, HT3, and HT4, that had the ability to degrade cellulose and cyanide. Based on the ability to degrade cellulose as indicated by the clear zone produced, colony zone, and the cellulolytic index (Table 1), The average cellulolytic index for 4 is from 0.82-2.06. Table 2 shows that isolate HA1 has the highest cellulolytic index is 2.06, followed by HB2, HT3, and HT4 with each cellulolytic index of 1.89; 1.75, and 0.82.

The ability of four isolates to degrade cyanide as seen in Table 2, showed of clear zone and colony zone that HA1 and HT4 isolates have higher clear zone values and so the cyanolytic index value of HA1 and HT4 also higher than the other isolates with cyanolytic index value are 1.03 and 1.0, while the other isolates only have a cyanolytic index lower than 1, it depend on the clear zone value and colony zone value.

Apart from the clear zone, enzyme activity is also important for assessing degradation ability. Table 3 shows the activity of the cellulase enzyme and B glucosidase enzyme in the four isolates obtained which were isolated from cassava waste (HA1, HB2, HT3, and HT4). Activity The cellulase enzyme as an indicator of cellulose degradation ability in the HA1 isolate was the highest, namely 7.58U/mL, and HT4 had the lowest cellulase enzyme activity value of 2.32 U/mL. The activity of enzyme β-glucosidase as an indicator of the high ability to degrade cyanide was produced by isolates HT4 and HB2 are 1.15 and 0.95U/mL

The characterization of cellulose and cyanide-degrading isolates can be seen in the Table 4. All isolates are gram-positive bacteria in the form of bacilli with different strains each because based on their characteristics, none of them are the same. The characteristics tested include shape, colony color, gram, aerobic/anaerobic properties, SIM (Sulfide, Indole, Motility) urease, citrate, and carbohydrate fermentation so that bacterial strains are produced that HA1=bacillus sp1, HB2=bacillus sp2, HT3=bacillus sp3 and HT4=bacillus sp4. The morphology and color of isolates were isolated from cassava waste consisting of HA1, HB2, HT3, and HT4 after being tested and observed under a microscope can be seen in Fig. 1. In Fig. 1, it can be seen that all bacteria are bacill with a purple color.

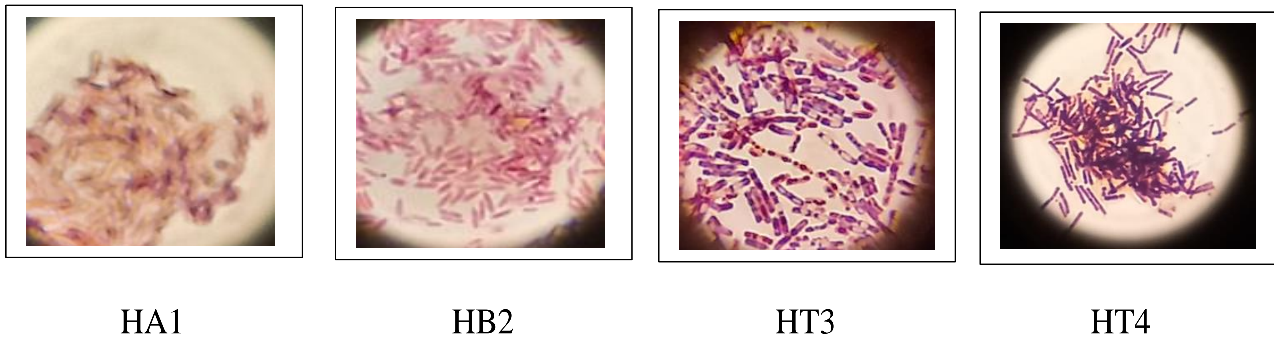

Fig. 1: Morphological form of soil isolate.

Table 1: Clear zone and cellulolytic index of cellulose degrading bacteria

Isolate	Clear Zone (mm)	Colony Zone (mm)	Cellulolytic Index
HA1	5.50	1.80	2.06
HB2	1.30	0.45	1.89
HT3	1.10	0.4	1.75
HT4	2.00	1.10	0.82

Table 2: Clear zone and cyanolytic index of cyanide degrading bacteria

Isolate	Clear Zone (mm)	Colony Zone (mm)	Cyanolytic Index
HA1	7.00	3.00	1.03
HB2	5.00	3.00	0.67
HT3	5.00	3.50	0.44
HT4	6.00	3.00	1.00

Table 3: Cellulase and β-glucosidase activity isolate from cassava waste

Isolate	Cellulase activity (U/mL)	β-Glukosidase activity (U/mL)
HA1	7.58	0.78
HB2	3.90	0.95
HT3	4.50	0.81
HT4	2.32	1.15

Table 4: Morphological, physiological and biochemical characteristics of isolates

Karakteristik	Isolates			
	HA1	HB2	HT3	HT4
Form	bacil	Bacil	bacil	bacil
spore	-	-	spore	-
Colour colonies	yellowish white	yellowish white	yellowish white	yellowish white
Gram	+	+	+	+
Aerob/Anaerob	Aerob	Aerob	Aerob	Aerob
Mortilitas	+	+	+	+
Urease	-	+/-	-	-
Citrate	-	-	-	-
Lactose	-	-	-	-
Glucose	-	+	-	-
Sucrose	-	+	-	-
Manitol	-	+	-	-
Nitrate	-	+	+	+
MR	-	-	-	-
VP	+	+	-	-
Indole	-	-	-	-
H2S	-	-	-	-
Gas	-	-	-	-
Strain	Bacillus sp1	Bacillus sp2	Bacillus sp 3	Bacillus sp 4

DISCUSSION

Cellulose and Cyanide-Degrading Bacteria Isolated from Cassava Waste

The isolation of cellulose and cyanide-degrading bacteria from cassava waste was carried out using the pour plate method on selective media containing CMC and KCN. This resulted in the identification of four isolates capable of effectively degrading both cellulose and cyanide, as evidenced by the clear zones produced in the selective media after 24 hours incubation at 37°C. These isolates were designated as HA1, HB2, HT3, and HT4. Cellulolytic bacteria play a crucial role in decomposing cellulose, a primary carbon source derived from photosynthesis, in lignocellulosic biomass. The decomposition of cellulose involves the hydrolysis of β-1,4 glycosidic bonds (Grata 2020). The variations in the clear zones produced by these isolates influence the cellulolytic index, which is depicted in Table 1. The cellulolytic index serves as a quantitative measure of an isolate's cellulose-degrading capability, effectively describing cellulolytic activity by quantifying the clear zone formation in CMC media (Ferbiyanto et al. 2016).

The research results demonstrate that isolate HA1 exhibited the largest clear zone and the highest cellulolytic index, which was measured at 2.05. This observation signifies that isolate HA1 excels in cellulose degradation, given its relatively high cellulolytic index. According to the classification criteria, a cellulolytic index is considered high if it exceeds 2, moderate if it falls within the range of 1-2, and low if it is below 1 (Choi et al. 2005). Based on the clear zone and the cellulolytic index data obtained, it is evident that the HA1 isolate outperforms the others, showcasing superior cellulose-degrading capabilities.

The clear zones produced on cyanide-selective media by each isolate isolated from cassava waste are presented in Table 2. Examination of Table 2 reveals that the HA1 isolate generated a larger clear zone, resulting in a higher cyanolytic index compared to the other isolates. This observation underscores the superior cyanide degradation capacity of the HA1 isolate. Cyanide-degrading bacteria utilize cyanide and its derivatives as nitrogen sources for growth, making them suitable for bioremediation processes. These bacteria possess metabolic pathways for cyanide degradation and mechanisms to counteract cyanide poisoning, such as alternative oxidases that are not susceptible to cyanide inhibition (Maria et al. 2021).

Cellulase and β-Glucosidase Activity by Isolates from Cassava Waste

Cellulase enzyme plays a pivotal role in cellulose degradation and the higher the cellulase enzyme activity, the greater the isolate's ability to reduce crude fiber content. In our study results, as displayed in Table 2, isolate HA1 exhibited the highest enzyme activity compared to the other isolates, with an enzyme activity of 7.58U/mL. This observation correlated with the larger clear zone and higher cellulolytic index exhibited by isolate HA1 compared to the other isolates. Cellulase activity is quantified in international units, denoted as units for milliliter (U/mL). A single unit corresponds to the quantity of enzyme required to break down 1μmol of cellulose into reduced sugars per minute under specified test conditions. The quantification of reduced sugar levels resulting from cellulose hydrolysis with cellulase enzymes is determined based on absorbance values at λ 540nm (Duza and Mastan 2013). The production of cellulase enzymes varies among cellulolytic bacteria, contingent upon the bacterial type and strain (Mohammadi et al. 2022). Cellulase important important in animal feed industry, it can used for feed processing biotechnology to improve the nutritional value of feed animals mainly the feed of agricultural waste or by-products. Cellulase degraded antinutional feed such as cellulose, oligosaccharides, β-glucan, pectins, lignin, inulin, dextrins, and arabinoxylans which ultimately improve the nutritional value of feed and animal health (Ejazz et al. 2021).

The β-glucosidase enzyme plays a pivotal role in hydrolyzing cyanogenic glucosides found in cassava waste, subsequently releasing cyanic acid, which later evaporates. Therefore, higher activity of β-glucosidase enzymes can lead to more effective reduction of cyanide content in cassava waste. The results revealed that isolate HT4 exhibited the highest activity of β-glucosidase enzymes, amounting to 1.15U/mL. The β-glucosidase enzyme is involved in the hydrolysis of cyanogenic glucosides, such as linamarin, which is enzymatically converted into BD glucopyranose and sinohydrin acetone. Cyanohydrin is subsequently transformed into acetone and cyanide. Cyanide, aided by various enzymes including cyanase, cyanide hydrotase, and cyanide dihydrotase, is further converted into CO_2, ammonia, and formic acid, which are essential for metabolic activities and bacterial growth (Gupta et al. 2009). Cyanogenic gylocosidase as a cyanide producer needs to be hydrolyzed by β -glucosidase, enzymatic hydrolysis of cyanogenic gylocosidase found in plants such as cassava is important to increase to reduce the cyanide content in cassava significantly because cyanide can have fatal effects on humans or livestock so that the activity of the β-glucosidase enzyme have important to reduce of cyanide content (Zhong et al. 2021).

Biochemical and Morphological Identification of Cellulose and Cyanide Degrading Isolates from Cassava Waste

The isolates obtained from the isolation process, totaling four isolates, underwent both biochemical and morphological identification to ascertain the nature and classification of each bacterium as an isolate. The results of biochemical identification are presented in Table 4 below. Table 4 illustrates that all four bacterial isolates are gram-positive (+), characterized by a purple coloration (Fig. 1). These isolates assume a bacilli morphology, with distinct strains for each isolate. In the Gram staining process, gram-positive bacteria exhibit a purple or blue coloration due to the low lipid content in their cell walls. When alcohol is introduced during the staining process, dehydration occurs, leading to a reduction in pore size. This, in turn, retains the dye, resulting in the observed blue or purple coloration. In contrast, gram-negative bacteria appear red (Sutari and Wayan 2020).

All four isolates are aerobic, signifying that these bacteria require oxygen to thrive and proliferate. These isolates, derived from cassava waste, are characterized as motile bacteria. Bacteria exhibiting positive motility possess flagella, enabling them to move. The ability of these bacteria to grow beyond the initial puncture or planting area was evident. The motility test serves the purpose of assessing a bacterium's capability to move (Ardiansyah et al. 2018). Positive motility in bacteria is indicative of the presence of flagella, an essential external component facilitating cellular movement (Sandi 2010).

Among the four isolates, isolate HT3 was identified as a spore-forming bacterium. Spore-forming bacteria possess an exceptional ability to endure and protect themselves in harsh conditions, including extreme temperatures, exposure to specific chemicals, and even competition with other parasitic bacteria. Bacillus species, which are known for their spore-forming capability, demonstrate relatively high resistance to physical and chemical treatments (Cho and Myong 2020). The urea test was conducted on the bacterial isolates, revealing that isolate HB2 exhibited urease activity during the incubation process, as indicated by the development of a pink coloration after 24 hours. In contrast, the other three isolates did not demonstrate urease activity. Urease is an enzyme produced by ureolytic microorganisms which hydrolyzes urea into ammonia and carbon dioxide. Microbial urease has been using to applications in biotechnology, agriculture, medicine, construction, and geotechnical engineering because of their ability to produce urea and calcium (Mekonnen et al. 2021).

Furthermore, the citrate test was employed to evaluate the isolates' ability to utilize nitrate as a carbon source, resulting in the production of sodium carbonate, an alkaline compound (Mahmudah et al. 2016). However, all four isolates in this study were unable to utilize citrate as a carbon source, yielding negative results. This was evident from the absence of a color change in the citrate medium following the addition of the bromothymol blue indicator; the medium remained green. Conversely, isolates capable of using citrate as a carbon source would cause the medium to turn blue.

The ability of isolates to ferment various carbon sources, including lactose, glucose, sucrose, and mannitol, was assessed. This research shows that all isolate can not fermentation of lactose, but The results indicated that only one isolate, HB2, was capable of fermenting these carbon sources, such as glucose, and sucrose. This means that the HB 2 isolate can utilize glucose and sucrose for growth but cannot use lactose as a carbon source. This fermentation was evident during incubation as indicated by the change in medium color from red to yellow, signifying acid formation (Giyatno and Endah 2020). Carbohydrate fermentation is a metabolic process that some

microorganisms use to break down substrates such as glucose and other sugars when O_2 is not available or cannot be used by the microorganisms. Fermentation includes glycolysis reactions (in which one glucose molecule is split into 2 pyruvate molecules), as well as additional reactions that produce various final products (acids, alcohols, gases) and fermentation is mainly a mechanism for regenerating NAD+ when the respiratory process does not occur (Petersen et al. 2021).

The research shows that of H_2S and gas test results were negative, which means that during fermentation the isolate did not produce H_2S and gas, this is indicated by the absence of black color and gas formation in the medium. The results of the MR-VP test in this study yielded negative outcomes, as the final color produced was brownish yellow rather than red or pink. This indicates that the isolate in question was unable to oxidize glucose to produce high concentrations of acid. A positive MR-VP test results in a final color of red or pink (Falloa and Yuni 2016).

Conclusion

Isolation and screening of cellulose-degrading and cyanide-degrading bacteria from cassava waste, based on the clear zone formation and the production of cellulase and β-glucosidase enzymes, yielded four isolates: HA1, HB2, HT3, and HT4. These isolates were identified as gram positive bacilli with the strain of each bacteria are bacillus sp1, bacillus sp2, bacillus sp3 and bacillus sp4.

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Author's Contribution

Hera Dwi Triani,Yetti Marlida, Ahadiyah Yuniza, Husmaini and Wulansih Dwi Astuti developed concept, conducted experiments, analyzed the data, and write to script. All authors approved the final version of the manuscript.

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