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Characterization of Banana Peel Waste Fermented by Lactobacillus casei and Its Antibacterial Potential

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ABSTRACT

The increasing global demand for energy and the depletion of fossil fuel reserves have encouraged the exploration of renewable and sustainable energy resources. This study aimed to isolate and characterize lactic acid bacteria (LAB) with potential applications in bioelectricity generation and antibacterial activity. The study involved bacterial isolation and enumeration; morphological and biochemical characterization; pH tolerance testing; antibacterial activity assays against several pathogenic bacteria; bioelectricity measurements with different electrode combinations (Zn/Pb, Zn/Cu, and Pb/Cu); and functional group identification using Fourier Transform Infrared Spectroscopy (FTIR). The main parameters evaluated were bacterial colony count, morphology, Gram characteristics, pH tolerance, inhibition zone diameter, electrical voltage production, and functional groups of bioactive compounds. The results demonstrated that the LAB population reached 3×10^7 *CFU/mL, indicating a high bacterial density with the potential to contribute to electrical energy production. The isolates exhibited colonies ranging from round to irregular shape, yellowish-white pigmentation, convex elevation, bacillary morphology, and Gram-positive characteristics. The isolate exhibited good growth at pH 6 and 7, moderate growth at pH 8 and 10, and no detectable at pH 4. The antibacterial activity varied among test organisms, with the highest inhibition against S. typhi (12 mm), followed by S. aureus (11 mm), while E. coli, K. pneumoniae, S. epidermidis, and S. pyogenes showed inhibition zones of approximately 10 mm. Bioelectricity measurements revealed average voltages of 0.4, 0.8, and 0.2 mV for the Zn/Pb, Zn/Cu, and Pb/Cu electrode pairs, respectively, with the Zn/Cu electrode pair producing the highest electrical output. FTIR analysis revealed a prominent absorption peak at 3326.67* cm^{-1}*, suggesting the presence of hydroxyl (-OH) and amine (-NH) groups associated with phenolic, flavonoid, alkaloid, and protein compounds. In conclusion, the isolated lactic acid bacteria demonstrated tolerance to a wide pH range, moderate antibacterial activity against several pathogenic bacteria, and the ability to generate bioelectricity, particularly when combined with Zn/Cu electrodes. The presence of bioactive functional groups further suggests their potential applications in renewable energy production and antimicrobial biotechnology.*

Keywords: Optimization, Banana peel, Lactobacillus casei, Biomass

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INTRODUCTION

Electrical energy is a form of energy associated with electric current and voltage that is used to operate mechanical equipment and generate other forms of energy, used to drive mechanical equipment and produce other forms of energy. Renewable energy is derived from natural resources that can be replenished continuously. Waste refres to discarded material generated by human activities, whether on a household, industrial, or mining scale (D'Souza, 2010). Garbage or household waste is waste generated by daily household activities, excluding feces and specific waste (He et al., 2012). Microbial biosensors consist of transducers coupled with living or immobilized microbial cells. Microbial biosensors have been widely applied can already be identified using genetic engineering (Malvankar et al., 2014), because microorganisms enable rapid, accurate, and sensitive detection of target analytes, and sensitive target analyte detection transducers in various fields such as medicine, environment, defense, food, and security (Hasibuan, 2016).

Nanowire networks provide electrical conductivity to the Geobacter biofilm, enabling the conversion of organic compounds into electricity in the MFC, and Enhanced nanowire production has been reported as one of the genetic modifications capable of improving current generation for current production. Microbial communities play important roles in biogeochemical processes. Microbial electrocatalysis relies on microorganisms that catalyze electrochemical reactions at electrode surfaces as catalysts for reactions that occur at the electrodes (Sunarsih (2018); Lei (2006); Hidayat et., al (2020). Amylose contributes to firmness, whereas amylopectin contributes to stickiness (pera) while amylopectin causes sticky properties. Amylose contributes to gel formation, whereas amylopectin imparts viscoelastic properties, while amylopectin forms viscoelastic properties (Schimel et al., 2012).

Lactic acid bacteria possess several unique characteristics, including the ability to survive in the presence of high levels of sugar, salt, and alcohol, and to ferment monosaccharides and disaccharides. These bacteria exhibit several characteristics, including: it lacks porphyrins and cytochromes, is catalase-negative, do not perform electron transport phosphorylation, and derive energy primarily through substrate-level phosphorylation. Based on their fermentation pattern, *Lactobacillus* is classified as a heterofermentative

bacterium, namely one that produces ethanol and other compounds, such as carbon dioxide, from glucose fermentation.

Glucose ⟶ Lactic acid+ ethanol + CO_2

One approach to reducing energy consumption is the use of amylase enzymes is to use the amylase enzyme, which hydrolyze starch without the gelatinization process (raw starch). This enzyme is the most widely developed microbial enzyme (Kaneko et al., 2005). Microorganisms offer several advantages, including rapid growth, ease of genetic modification, and minimal space requirements are fast production times, ease of modification, and the lack of a large space requirement (Sun et al., 2008). The reaction proceeds through the formation of an oxocarbenium ion intermediate in the transition state, followed by the formation of a covalent intermediate. Subsequently, a water molecule attacks the covalent bond between the oxygen atom and the aspartic acid residue at position 193. Glutamic acid subsequently accepts a proton from the H_2O molecule, and the aspartic acid residue 193 forms a new hydroxyl group on the glucose molecule (Souza et al., 2010)

METHODOLOGY

The materials used were banana peels, distilled water, durian seeds, 70% ethanol, glacial acetic acid (Merck), DNS reagent, Magnesium powder, concentrated hydrochloric acid, Lactobacillus plantarum, n-Butanol (Merck), NaCl, NaOH, Nutrien broth medium (Merck), nutrient agar medium (Himedia, India), peptone water media, Mueller-Hinton (MH) medium, glycerol, spirit, Gram staining reagents, glass slide, Whatman paper no. 42 filter paper, label paper, wrap, aluminum foil, test bacteria (*Salmonella typhi, Staphylococcus epidermidis, Klebsiella pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, and Escherichia coli*), cotton, $FeCl_3$, ethanol, pH indicator, potassium dihydrogen phosphate (KH_2PO_4), Gram Staining, ammonium sulfate ($(NH_4)_2SO_4$), magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), sulfuric acid (H_2SO_4), sodium potassium tartarate (NaK-tartarate), sodium metabisulfite, 3,5-dinitrosalicylic acid (DNS reagent), 96% (v/v) ethanol, distilled water, glycerol, NaCl, NaOH, copper wire, agar (term to be confirmed), Pb, Cu and Zn electrodes.

The instruments used were 1000-µl micropipette, micropipette 10-100 µl, micropippete tips (blue and yellow tip), drop pipette, sample bottles, stir bar, beaker (pyrex), vacuum Buchner funnel, 250 mL glass funnel (pyrex), test tube, test tube rack, vortex, spatula, watch glass, blender, fermentation vessel, spray bottle, petridish, inoculating loop, analytical balance (Ohaus Pioneer PA214), oven, Hot plate, incubator, Bunsen burner, magnetic stirrer,

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Erlemenyer flask 250 mL (pyrex), colony counter, 1.5-mL and 2.0-mL Eppendrof microtubes, graduated beaker (if beaker is intended; if measuring cup is correct, retain as is), thermometer, sterile L-shaped spreader, autoclave, laminar flow, inoculating needle, pasteur pipette, hot plate magnetic stirrer, colony counter, vortex, eppendorf, micropipette, micro tip, heating block, spatula, oven, desiccator, shaker, analytical balance, labels, wrapping film sterile sample bottles, thermometer, pH meter, petri dishes, water bath shaker (Julabo Sw22), freezer, water bath (Griffin), incubator (Fisher), rotary shaker, compound microscope, stopwatch, bioreactor MFC dual compartment digital multimeter (DM-133D), analytical balance, cables and alligator clips. freezer, microscope, stopwatch, and a Fourier-transform infrared (FTIR) spectrometer.

Microbial optimization and screening were performed using *Salmonella typhi, Staphylococcus epidermidis, Klebsiella pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, and Escherichia coli,* to evaluate their potential for bioelectricity generation with MFC using household waste-derived substrates, particularly fermented banana peel pulp, with different electrode combinations, including Zn/Cu, Zn/Pb, and Pb/Cu electrode pairs.

RESULTS AND DISCUSSION

Banana peel pulp is a lignocellulosic biomass. Lignocellulose is one of the most abundant and renewable biomass resources on Earth. The conversion of lignocellulosic biomass into biofuels and value-added products has attracted increasing global attention as a means of producing biofuels such as bioethanol and other value-added materials. In this study, banana peel biomass was fermented using Lactobacillus casei to produce bioactive products with potential applications in bioenergy generation from banana peel biomass (lignocellulose) through a fermentation process using *Lactobacillus casei* starter to produce bioproducts that can increase product conversion efficiency.

Optimization and Screening of Banana Pulp Fermentation Using *Lactobacillus casei*

The fermented samples were serially diluted prior to colony enumeration to enumerate the colonies in the sample. Serial dilution was performed to obtain countable bacterial colonies is to minimize the number of microbes suspended in the liquid so that colonies that grow in the Petri dish containing the medium appear as single colonies. From the calculation of the number of colonies, the bacterial population reached 13 x 10^7 CFU/mL. This

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colony count indicates a high density of lactic acid bacteria of lactic acid bacteria are present in the sample, which may contribute to bioelectricity generation.

LAB, including *Lactobacillus plantarum*, *Lactococcus lactis*, and *Enterococcus faecalis*, are capable of generating bioelectricity through extracellular electron transfer (EET), In this mechanism, electrons generated during cellular metabolism are transferred to external electron acceptors, such as the anode of a microbial fuel cell during cellular metabolism are transferred from the intracellular environment to external electron acceptors such as the anode of a microbial fuel cell

Banana peel samples fermented with Lactobacillus casei underwent heterofermentative metabolism are among the lactic acid bacteria that undergo heterofermentation. Heterofermentative bacteria ferment glucose into lactic acid, CO_2, ethanol, and energy. The LAB used in this study exhibited several advantages, including the absence of toxic metabolite production, microaerophilic growth, aerotolerance, efficient sugar fermentation, and inhibition of putrefactive microorganisms, are microaerophilic and aerotolerant in simple fermentation processes, ferment simple sugars, and inhibit putrefaction. The heterofermentative pathway can be summarized as follows: the final result can be seen through the reaction equation process below:

Glucose + ADP + Pi ⟶ Lactic + Ethanol + CO_2 + ATP

Figure 1. Screening of bacterial isolates obtained from fermented banana peel samples.

Morphology Test of Banana Peel

The isolates were purified before morphological characterization in the Petri dish is then carried out by examining the sample's morphology on agar medium, both macroscopically and microscopically. Morphological observations were conducted qualitatively using both macroscopic and microscopic examinations and directly on Petri dishes containing bacterial colonies originating from banana peel pulp samples. Macroscopic observations with the naked eye The colonies were round to irregular in shape, yellowish-white in color, and exhibited a convex elevation, irregular, yellowish-white, and convex. These characteristics are consistent with those of lactic acid bacteria that the samples are lactic acid bacteria. Lactic acid bacteria are

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characterized by a round shape, a white color with a convex elevation, motility, anaerobic metabolism, catalase negativity, nonspore-forming, and growth in nutrient broth or agar medium (Sutrisna et al., 2013).

Representative colonies were further examined using Gram staining were also subjected to microscopic examination using the Gram stain. Gram staining is one of the most widely used techniques for bacterial identification and widely used techniques for identifying bacteria. The staining procedure involved crystal violet, iodine solution, alcohol as a decolorizing agent, and safranin as a counterstain in the Gram-staining process include crystal violet dye, iodine solution, an alcohol solution (a decolorizing agent), and a counter dye, safranin. Gram-positive bacteria retain the crystal violet stain, whereas Gram-negative bacteria are decolorized and subsequently stained with safranin, they will retain the crystal violet dye, whereas Gram-negative bacteria will lose it after being washed with fuchsin or safranin (Malvankar et al., 2014). Gram staining revealed rod-shaped, Gram-positive bacterial cells, the cell morphology of the banana peel pulp sample showed it was a bacillus, and it was Gram-positive.

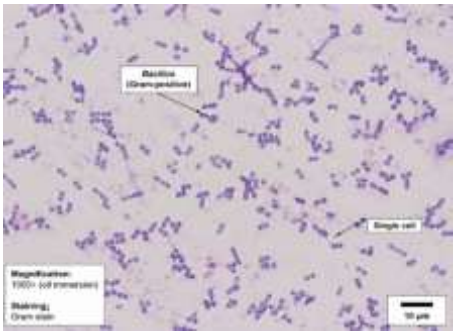

Figure 2. Gram-staining results showing the morphology of bacterial isolates.

Bacterial Tolerance Test of Banana Peel

Purified bacterial isolates were evaluated for their tolerance to different pH conditions was evaluated using a tolerance test with pH variations to determine the optimum pH for the microbial samples at each pH. The pH values evaluated in this study were 4, 6, 7, 8, and 10. These pH values were selected to represent acidic to alkaline conditions is based on the pH found in the remaining waste, which ranges from acidic to alkaline. The observations were as follows: at each pH, compared with the control (without bacteria), were: pH 4 (no visible growth); pH 6 (abundant growth); pH 7 (abundant growth); pH 8 (less cloudy); and pH 10 (moderate growth). These results indicate that, it can be concluded that the isolate were unable to grow in an acidic environment at pH 4. This is because overly acidic conditions will cause the LAB in the sample to die. In contrast to pH 6 and 7 (optimum pH), the isolates exhibited optimal growth in slightly acidic conditions. Moderate

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bacterial growth was still observed under alkaline conditions quite well at alkaline pH values, namely pH 8 and 10, because there are microbes that can live in such environments.

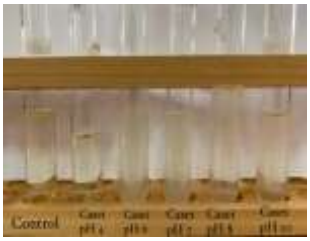

Figure 3. Growth of bacterial isolates under different pH conditions.

Potential Activity of Banana Peel Against Pathogenic Bacteria

Bacteriocins are proteins produced by certain bacteria that exhibit bactericidal activity against Gram-positive bacteria. Bacteriocins produced by lactic acid bacteria have potential applications as natural food preservatives because they inhibit foodborne pathogens, which contain high levels of carbohydrates, protein, and fat. Bacteriocins are classified into four major classes, namely class I bacteriocins, which are called lantibiotics. For example, nisin produced by Lactococcus lactis is highly active against most Gram-positive bacteria by damaging their cell membranes. Class II bacteriocins are non-lantibiotics, for example, pediocin produced by *Lactobacillus plantarum*, which inhibits *Listeria monocytogenes*. Class III bacteriocins are heat-labile proteins with a molecular weight of more than 30 kDa. Class IV bacteriocins are glycoproteins or lipoproteins (Rachmawati et al., 2005).

The antibacterial activity of LAB against pathogenic bacteria was evaluated using the agar diffusion (modified Kirby–Bauer) method with paper discs. This method is widely used to evaluate antimicrobial activity of an antibiotic found in banana peel pulp samples against disease-causing microorganisms, during which all active metabolite compounds produced by LAB in the samples are expressed. The pathogenic bacteria used in this study included, including *Salmonella typhi, Staphylococcus epidermidis, Klebsiella pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, and Escherichia coli*

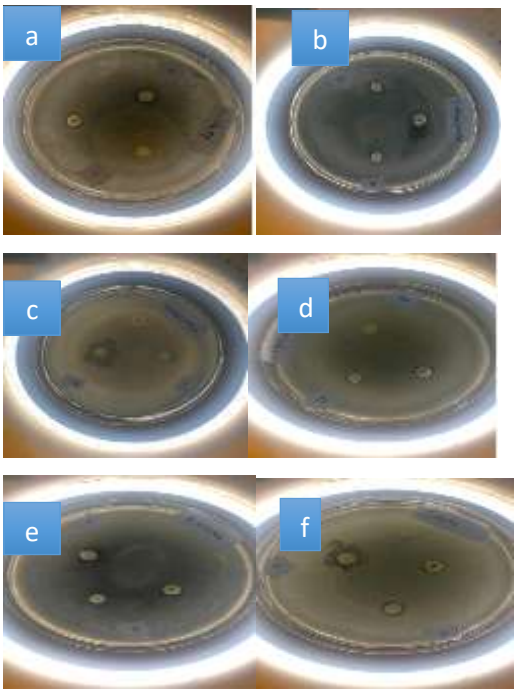

Figure 4. Antibacterial activity of fermented banana peel samples against pathogenic bacteria.

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Antibacterial activity was evaluated using cultures of lactic acid bacteria and their neutralized supernatants using cultures of lactic acid bacteria and their neutral supernatants. The culture of lactic acid bacteria in the banana peel pulp sample produced clear inhibition zones, indicating antimicrobial activity against the test bacteria, while the neutral supernatant, which also exhibited inhibitory activity, was from lactic acid bacteria that exhibit potential LAB activity beyond organic acids. The starter culture used in this study was *Lactobacillus casei*, This species can produce several enzymes, including amylase, β-glucosidase, cellulase, and phytase, β-glucosidase, and cellulase, as well as phytase, which can convert phytic acid into inositol and phosphoric acid. Phytase exhibits optimal activity at pH 5.0 and a temperature of 25-40 °C, The incubation temperature used in this study was 37 °C.

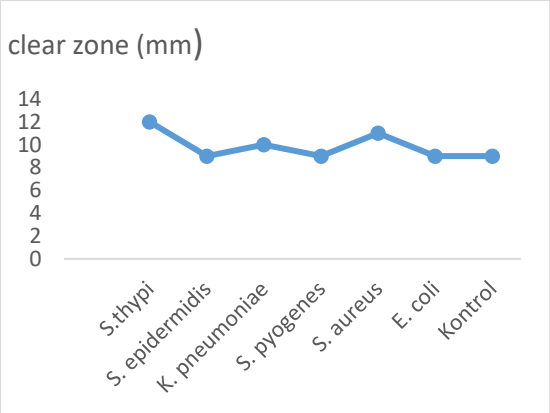

Figure 5. Antibacterial activity of LAB against pathogenic bacteria after 24 h of incubation.

The inhibition zone represents the area where bacterial growth was inhibited. A larger inhibition zone indicates stronger antibacterial activity, the more effective the antibacterial is against certain bacteria. *S. thyphi* exhibited an inhibition zone of approximately 12 mm., indicating a high level of antibacterial activity against this bacterium. *S. epidermidis*: An inhibition zone of approximately 10 mm was observed, which means its antibacterial activity is lower than that of *S. typhi*. similarly, *K. pneumoniae and S. pyogenes* exhibited inhibition zones of approximately 10 mm, indicating moderate antibacterial activity. *S. aureus* exhibited a slightly larger inhibition zone (~11 mm) indicating relatively stronger activity against the bacteria. *E. coli* had a clear zone of about 10 mm, indicating that the sample had similar antibacterial effects to *K. pneumoniae* and *S. epidermidis.* These results demonstrate differences in antibacterial activity among the tested bacterial species of samples against different types of bacteria. The fermented sample exhibited the strongest antibacterial activity against *S. typhi* and less effective against *S. epidermidis* and *E. coli.*

The inhibition zone formed by bacteriocins is clear, round, and broad. Lactic Lacid bacteria are known to produce antimicrobial compounds that inhibit spoilage microorganisms and pathogenic

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bacteria that inhibit other microorganisms, including food-spoilage organisms and pathogens. Antimicrobial compounds are produced during fermentation and remain in the food, inhibiting the growth of harmful and pathogenic bacteria (Rachmawati et al., 2005).

LAB Biomass Test as a Bioelectricity

The dual-chamber reactor contained nutrient broth supplemented with molasses as the microbial growth medium from Nutrient Broth as a medium for growing microbes, along with banana pulp samples. Molasses served as a carbohydrate-rich substrate. The microorganisms metabolized the substrate and generated protons and electrons and convert them into energy in the form of H^+ ions. The anode chamber was maintained under anaerobic conditions to prevent oxygen intrusion to prevent oxygen from entering. The cathode chamber contained 1 L of electrolyte solution with 1 L of water as an electrolyte solution and left open. Each chamber was equipped with one of the following electrode pairs: Zn/Pb, Zn/Cu, or Pb/Cu., a metal electrode pair (anode-cathode) is installed: Zn/Pb, Zn/Cu, or Pb/Cu. The electrode combinations were selected based on their electrochemical potential differences on the current generated when electrons flow externally through the circuit from the anode to the cathode due to the potential difference between the two electrodes in an electrochemical cell (Galvanic cell. Lead (Pb) can function as an electrode material because of its electrical conductivity and ease of fabrication as an electrode material in bioelectricity systems due to its good electrical conductivity and ease of fabrication into various electrode configurations. Additionally, Pb is relatively inexpensive and has been widely used in electrochemical systems, although its use should be carefully controlled because of its toxicity due to its toxicity to human health and the environment. The dual-chamber reactor was constructed from plastic containers connected by a PVC tube containing an agar–salt bridge, connected by a PVC pipe containing a salt bridge made from salt and agar.

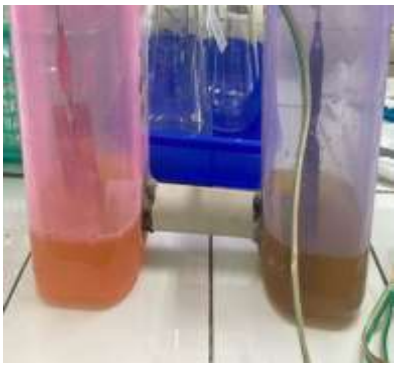

Figure 6. Schematic of the dual-chamber microbial fuel cell connected by an agar–salt bridge.

Voltage measurements were recorded every 5 min for 60 min using a digital multimeter using a DT-830B multimeter for 1 hour, with readings taken

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every 5 minutes, to determine the maximum voltage produced by 5 g of banana peel waste substrate. Electrical potential was measured using different electrode combinations using a variety of electrodes. Table 1 presents the measured voltages the results of electrical voltage measurements using Zn/Pb, Zn/Cu, and Pb/Cu electrodes under conditions before fermentation (6.0) and after fermentation (5.0).

Table 1. Results of Electrical Voltage Measurement Using an Electrode

Time	Voltage (mV)		
(minutes)	Zn/Pb	Zn/Cu	Pb/Cu
5	0,4	0,8	0,2
10	0,4	0,8	0,2
15	0,4	0,8	0,2
20	0,4	0,8	0,2
25	0,4	0,8	0,2
30	0,4	0,8	0,2
35	0,4	0,8	0,2
40	0,4	0,8	0,2
45	0,4	0,8	0,2
50	0,4	0,8	0,2
55	0,4	0,8	0,2
60	0,4	0,8	0,2

The electrodes functioned as conductors for electron transfer during the electrochemical reactions, a conductor of electric current (conductor), is used, using the principle of separating two redox reactions in the form of half an oxidation reaction at the anode and half a reduction reaction at the cathode. The average voltages recorded over the 60-min period were 0.4, 0.8, and 0.2 mV for the Zn/Pb, Zn/Cu, and Pb/Cu electrode pairs, respectively at each electrode (Zn/Pb, Zn/Cu, Pb/Cu), the average voltage is obtained over 1 hour (60 minutes), with measurement intervals of 5 minutes (0.4, 0.8, 0.2 mV). On the Zn/Cu electrode, the electrode that undergoes oxidation will form $Zn^{2+,}$ which then enters the solution. The reaction of the released electrons is captured by Cu^{2+} from the solution, so that a precipitate is formed. With the reaction equation as follows:

The electrochemical reactions are summarized below:

Anode: $Zn_{(s)} \rightarrow Zn^{2+}{}_{(aq)} + 2e$

Cathode: $Cu^{2+}{}_{(aq)} + 2e\text{-} \rightarrow Cu_{(s)}$

Overall cell reaction: $Zn_{(s)} + Cu^{2+}{}_{(aq)} \rightarrow Zn^{2+}{}_{(aq)} + Cu_{(s)}$

The substrate was oxidized by the bacteria, generating electrons and protons at the anode, which then produce electrons and protons at the anode. The electrons were transferred through an external circuit, whereas protons migrated through the proton exchange pathway toward the cathode through an external circuit cable, while protons are diffused through a proton

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exchange membrane (PEM) to the cathode. Protons (H^+ ions) and electrons in the cathode will then react with oxygen to form water (H_2O) (Logan, 2008).

Bioactive Compound Test Using FTIR

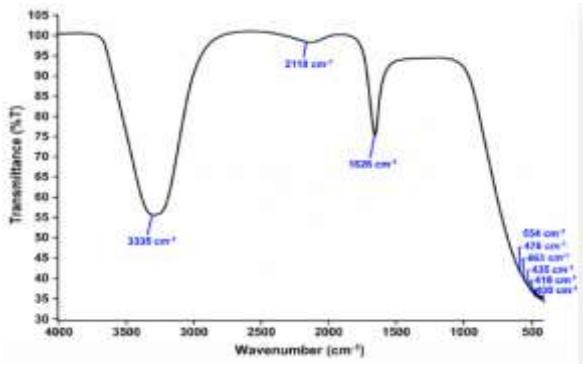

Figure 7. Bioactive Compound Result of FTIR

Figure 7 shows the FTIR spectrum of the fermented sample, which shows absorption peaks at several wavelengths (wavenumbers, cm^{-1}). Each absorption band corresponds to a characteristic functional group the presence of a particular functional group in the sample. An absorption band at 3326.67 cm^{-1} is commonly assigned to O–H and N–H stretching vibrations of 3326.67 $cm^{-1,}$ the peak around this area usually indicates the presence of a hydroxyl group (-OH), such as in alcohols or carboxylic acids. This band may also be associated with amine-containing compounds an amine group (-NH) in proteins or in nitrogen-containing polymers commonly found in flavonoids, alkaloids, or phenolic compounds. Hydroxyl groups are commonly associated with phenolic compounds and flavonoids in compounds with antioxidant activity, such as flavonoids and phenolics.

The absorption band at 2145.50 cm^{-1} in this area generally indicate triple bonds, such as nitrile groups (-C≡N) or terminal alkenes (-C≡C-). Nitrile groups are often found in alkaloids. Alkaloids are a group of secondary metabolites that typically contain nitrogen and exhibit high biological activity, including analgesic, anticancer, and antibiotic properties. The absorption band at 1634.61 cm^{-1}, this peak is often associated with carbon-carbon double bonds (C=C) in alkenes or carbonyl groups (C=O) in peptide bonds (proteins), ketones, or amides. At this wavelength, the presence of flavonoids, terpenoids, or quinones. Flavonoids have aromatic rings with C=C double bonds, while terpenoids often contain C=C double-bond structures. Peaks below 500 cm−1 usually indicate vibrations from metal groups or heavy atoms, which may originate from metal-oxygen or metal-halogen bonds. Overall, the FTIR spectrum suggests the presence of several bioactive functional groups, the sample likely contains secondary metabolites such as flavonoids, due to the presence of –OH groups and C=C double bonds; alkaloids, due to the presence of the typical nitrile group (-C≡N); and terpenoids, due to the presence of C=C double-bond peaks.

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CONCLUSION

In conclusion, the isolated lactic acid bacteria exhibited good pH tolerance, antibacterial activity, and the ability to generate bioelectricity good pH tolerance, antibacterial activity, and the ability to generate electrical potential. The presence of amine (-NH) and hydroxyl (-OH) functional groups suggests the occurrence of bioactive compounds that may contribute to both antimicrobial and electrochemical activities. These findings highlight the potential application of lactic acid bacteria in sustainable biotechnology and renewable energy systems the dual potential of lactic acid bacteria as both antibacterial agents and bioelectricity-producing microorganisms, suggesting their promising applications in sustainable biotechnology and renewable energy development.

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