OPEN ACCESS

World Journal of Multidisciplinary Studies
ISSN: 3049-0855 | Vol. 2, No. 1, 2025
Website: https://wasrpublication.com/wjms/

ISOLATION AND CHARACTERIZATION OF BENEFICIAL MICROBES FOR MUNICIPAL SOLID WASTE MANAGEMENT

Ofunwa, J.O[1]., Egurefa, S.O[2]., Awari, V.G[1]., Umeoduagu, N.D[1]., Egwuatu, C.I[3]., Orji, C.C[4]., Ifediegwu, M.C.[4], Anazodo, C.A[4]., Ojeah, I.K[5]. and Ebo P.U[1].

[1]Depertment of Microbiology, Tansian University, Umunya, Anambra, Nigeria

[2]Department of Science Laboratory Technology, Southern delta University PMB 05, Ozoro Delta State, Nigeria

[3]Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka, Nigeria.

[4]Department of Applied Microbiology, Nnamdi Azikiwe University, P.M.B. 5025 Awka, Nigeria

[5]Microbiology Department, University of Delta, Agbor P.M.B 2090, Agbor, Delta State, Nigeria.

Received: 18/11/2024 | Accepted: 28/12/2024 | Published: 21/01/2025

Abstract: Developing an eco-friendly method of solid waste management in society has been challenging. This study was carried out to isolate and characterize beneficial microbes for municipal solid waste management. Soil and root soybean samples were collected at a garden located at Chukwuemeka Odumegwu OJukwu University, Uli Campus. Ripe queen pineapple (Ananas comosus) specimens were bought from Nkwo Ogbe Market, Ihiala, Anambra State. The samples were screened for beneficial microorganisms using a composed medium for bacteria and yeast extract agar for yeasts. The ability of the microbial isolates to degrade complex environmental wastes was investigated using starch, fat, protein, lignin, and cellulose-containing media, which also enabled clear zones and enzymatic indexes to be measured using Congo red stain. The beneficial microbes were characterized using cultural, microscopic, biochemical, and molecular features, and phylogenetic relatedness was also investigated using standard microbiological techniques. The results revealed that 13 microorganisms were isolated from the cultured substrates, and three of the 13 isolates exhibited high degradative potential, as shown in the value of the enzymatic index. These beneficial microbes were identified as Enterobacter sp. RB1 (KX548942.1), *Bacillus thuringensis* PSB2c (ON939710.1), and Candida tropicalis Y3 (KY112741.1). The Phylogenetic relationship depicted that *Bacillus thuringensis* PSB2c and *Candida tropicalis* Y3 had similar ancestry, while Enterobacter sp. RB1 deviated in ancestry This study has shown that beneficial microbes degrade complex organic matter in solid wastes found in the environment, which could be optimized for novel solid waste management.

Keywords: Solid Waste Management, Beneficial Microorganisms, Biodegradation, Enzymatic Index, Microbial Characterization, Phylogenetic Analysis, Eco-friendly Solutions.

1.0 Introduction

Solid wastes predominantly comprise domestic wastes, with sometimes the addition of commercial wastes collected by a municipality within a given area (Bernandi et al., 2018). Solid wastes generated in the urban area are collectively known as municipal solid wastes, while solid wastes generated in villages are collectively called rural solid wastes. It is worth noting that most solid wastes are generated in urban areas due to large settlements and commercial activities (Ezugworie et al., 2023). Most of these solid wastes emanate from household items, including the remains of agricultural products and other living things.

Research has revealed that waste generation is part of man's lifestyle and can never be changed because the generation of some waste cannot be avoided (Gautam et al., 2012). Solid waste disposal and management have been difficult due to the negligence and nonchalant attitude of individuals in the community. Indiscriminate disposal of refuse has been adopted by most households, especially during the rainy season, because it is easy to practice with disadvantages (Jain et al., 2018). Open dumping of solid waste causes several environmental challenges, such as pollution and erosion. Some individuals have devised means of solid waste management such as open burning and dumping in water bodies, which create more threats to man and the ecosystem, as Jurado et al. (2014) report.

Researchers have been accelerating efforts for an alternative means of waste management that could be friendly to the ecosystem and cost-effective (Karanja et al., 2019). It is worth noting that some individuals have succeeded in recycling some solid wastes into a form that could be beneficial to man, though the byproduct and the processes involved in the recycling are capable of generating harmful substances for the environment. This indicates that some methods that have been employed in recycling waste are not eco-friendly (He et al., 2013).

Environmental cleanup through bioremediation relies on the action of naturally occurring microorganisms for decontamination. Biodegradation, as defined by (Orji et al., 2014; Ifediegwu et al.,

***Corresponding Author**
Ofunwa, J.O.*
Depertment of Microbiology, Tansian University, Umunya, Anambra, Nigeria.

2015; Agu et al., 2017; Ojiagu et al., 2018), is the biological process by which living microorganisms convert organic waste into nutrients. A study by Kashyap et al. (2019) stated that microorganisms break down complex materials in biodegradable solid wastes. These microorganisms' potentials have not been fully tapped globally due to inadequacy in research. When microorganisms degrade complex organic matter in solid wastes, the degradation product has been proven to be eco-friendly as the microbes optimize the products such as carbon, nitrogen, and phosphorus for metabolism (Gautam et al., 2012). The recent surge of interest in organismal peroxidases stems from their compelling catalytic advantages, such as their high biocompatibility, biodegradability, biodigestibility, simple preparation, and stability under a wide array of physicochemical conditions (Oparaji et al., 2024).

Several studies have been documented on beneficial microorganisms that are capable of degrading solid wastes, such as Jurado et al. (2014), Jain et al. (2018), Karanja et al. (2019), and Kashyap et al. (2021), but few works are available on the isolation and characterization of beneficial microbes for municipal solid waste management. Hence, this study aims to isolate and characterize beneficial microbes for municipal solid waste management. The outcome of this study would be essential in developing novel solid waste management strategies, especially in urban areas.

2.0 Materials and Methods

Sample Collection

Rhizospheric soil samples and roots of soybeans were aseptically collected with a sterile hand trowel and knife from the school garden (coordinates describe using hand-held GPS) within the premises of Chukwuemeka Odumegwu Ojukwu, Uli Campus, Ihiala Local Government Area, Anambra State. Ripe queen pineapple (Ananas comosus) specimens were bought from Nkwo Ogbe Market, Ihiala, Anambra State. All the samples were placed into sterile polyethylene bags and transported on ice to the Microbiology Laboratory of Chukwuemeka Odumegwu Ojukwu University Uli Campus, Nigeria, for further analysis.

Isolation of Rhizospheric Bacterial (RB) Species

According to the method of Ogbo and Okonkwo et al. (2012), the washed roots of soybean plants were cut into 3 cm segments and disinfected by soaking in 70 ethanol for 5 min, in 6.25% sodium hypochlorite for 10 min, followed by several rinses in sterile distilled water. Intact root pieces (0.5 to 1.0 cm) were then placed into tubes of semisolid (0.05%) nitrogen-free biotin medium (NFb) and incubated without shaking for 5 days at 30℃. This medium was composed (g/L) of: DL-malic acid 5; KOH 4; K_2HPO_4 0.5; $MgSO_4\cdot 7H_2O$ 0.2; $CaCl_2$ 0.02; NaCl 0.1; $FeSO_4\cdot 7H_2O$ 0.5; Agar 5 g; and (mg/L) of: $NaMoO_4\cdot 2H_2O$ 2; $MnSO_4\cdot H_2O$ 10. The medium also contained 2 mL of a 0.5% solution of bromothymol blue in 95% ethanol and had a final pH of 6.8. After incubation, the white pellicles from tubes that showed this characteristic feature were subcultured onto solid, semi-selective, Congo red-NFb medium. The appearance of a red to scarlet colony suggestive of the rhizospheric bacterium was selected and purified by repeated streaking on the same medium.

Isolation of Yeast

The fruits were washed with sterile disinfectant water, drained, peeled, and sliced into smaller pieces. The juices were aseptically extracted using a hand juice extractor, filtered with a muslin cloth, and the filtrates collected in a sterile plastic container as described by Umeh et al. (2019). For the isolation of the yeasts, 100 mL of Yeast Extract Dextrose Peptone Broth (40 g of peptone water, 10 g of yeast extract, and 20 g of dextrose sugar (sucrose) in 1 L of distilled water) was added into 250 mL conical flask containing 100 g of the 48-h fermenting crushed pineapple, respectively, and were incubated for 2–5 days at 30 °C to enhance microbial growth. An aliquot of 0.1 mL of the YEDP broth containing the pineapple juice was inoculated onto Potato Dextrose Agar (PDA) medium (50 mg/L of tetracycline and 0.05 mg/L of gentamycin were added to the PDA medium to inhibit bacterial growth) in duplicates using a glass spreader. The plates were incubated at 30 °C for 72 h. The colonies that appeared on the plates were further subcultured and incubated for another 48 h at 30 °C to obtain pure cultures. The selected yeast isolates were obtained, and the pure cultures of the isolates were stored in 10% glycerol at 4 °C in Bijou bottles (Alabere et al. 2020).

Screening of Strains with High Degradative Activity

According to the methods of Zhao et al. (2017) and Limaye et al. (2017), the dominant strains were inoculated (100 µL) into starch, fat, protein, lignin, and cellulose-containing media. The starch medium (1000 mL) comprised soluble starch 2%, NaCl 0.5 %, peptone 0.5 %, and agar 2%; the fat medium (1000 mL) comprised peptone 1%, NaCl 1%, $CaCl_2\cdot 7H_2O$ 0.01%, Tween-80 1%, and agar 2%, pH 7.4–7.8; the protein medium (1000 mL) comprised non-fat dried milk 5% and agar 1.8 %; lignin medium (1000 mL) comprised $(NH_4)_2SO_4$ 0.2 %, K_2HPO_4 0.1 %, KH_2PO_4 0.05 %, $MgSO_4$ 0.02 %, yeast extract 0.1 %, maltose 0.02 %, asparagine 0.02 %, agar 2.3 % and 0.01 % guaiacol dissolved in ethanol. Sterile trace solution ($CuSO_4\cdot 7H_2O$ − 0.01 %, $ZnSO_4\cdot 7H_2O$ 0.03 %, $MnCl_2\cdot 5H_2O$ 0.02 %, $MgSO_4\cdot 7H_2O$ 0.25 %, $CaCl_2\cdot 2H_2O$ 0.15 %, $FeSO_4\cdot 7H_2O$ 0.03 %, and $COCl_2\cdot 6H_2O$ 0.01 %) 0.1 % v/v was added to the lignin medium after autoclaving, and the cellulose medium (1000 mL) comprised K_2HPO_4 0.1 %, carboxyl methyl cellulose 0.5 %, $MgSO_4$ 0.05 %, glucose 0.1 %, yeast extract 0.05 %, $NaNO_3$ 0.1 %, KCl 0.1 % and agar - agar 2.2 %. All the plates were incubated for 5 days at room temperature. The presence of “clear zones” surrounding the colonies was taken to indicate degradative activity. Moreover, iodine was added to the starch medium and neutral red dye to the fat medium to increase the color contrast. The lignin medium will be covered with 0.5% methyl orange dye solution, while the cellulose agar surface will be covered with 0.1% Congo red solution for 15 minutes. Excess Congo red will be poured off, and the plate will be overlaid with 1 M NaCl for 1 h. The degradative activity will be measured in triplicate and the mean will be calculated. Strains with the greatest degradative activity were selected to comprise the consortium (effective microbes).

Characterization and Identification of the Selected Microbial Isolates

The four strains with the greatest degradative activities were selected and identified further by colonial morphology, microscopic technique, biochemical tests, and molecular methods using the methods of Willey et al. (2008), Cheesbrough (2006), and HPA (2007).

Morphological characteristics

Colonial morphology

After subculturing and incubation, colonial morphological properties such as shape, elevation, margin, optic, texture, color, size, and surface characteristics of the selected bacterial strains were observed and noted (Willey et al., 2008).

Microscopic morphology

Gram staining

This technique divides bacteria into Gram-positive and Gram-negative groups. A smear of the isolate was made on a clean, dry, grease-free slide using a sterile wire loop. The smear was air-dried and heat-fixed by passing over flame quickly three times. It was then covered with a 0.5% crystal violet solution for 1 minute and rinsed with distilled water. The slide was flooded with 1% Lugol’s iodine (which served as a mordant that fixes the dye inside the cell). The iodine was washed off after 1 minute, and 95% ethanol was used to decolorize the smear for 30 seconds. The smear was counter-stained with a 0.1% safranin dye solution for 1 min. It was then washed off the slide air-dried and observed under the microscope using an oil immersion objective lens after placing a drop of oil immersion. Gram-positive and negative reactions were indicated by purple and red colors, respectively (Cheesbrough, 2006).

Spore staining

According to the HPA (2007) method, smears of the isolates were prepared and fixed on a slide. The underside was vapor heated and flooded with a 5% malachite green solution. Heating continued until visible water condensate formed under the slide with evaporation at the top. It was washed using distilled water. Smears were counterstained with 0.5% safranin solution for 10 seconds. Slides were washed, dried, and observed under an oil immersion objective lens after placing a drop of immersion oil. A green space in the cells would indicate the presence of spores.

Biochemical characteristics

Catalase test

Cheesbrough (2006) stated that the test identifies organisms that produce the enzyme catalase. A drop of 30% freshly prepared hydrogen peroxide (3 mL $H2O2$ in 7 mL $H2O$) was placed on a clean slide, and a loopful of isolate was transferred into it and emulsified. The appearance of gas bubbles indicates a positive reaction. The reagent was shaken before the test to expel dissolved oxygen and avoid a false positive result.

Indole test

Cheesbrough (2006) stated that the tryptone broth was prepared, and 5 mL was dispensed into each test tube and sterilized. The isolates were inoculated into the test tube and incubated at 28 °C for 48 hr. After incubation, 5 drops of Kovac’s reagent (4-p-dimethyl-aminobenzaldehyde) were added to the tubes, shaken gently, and allowed to settle. A red coloration in alcohol dye indicates a positive result for the reaction.

Motility test

The organism's directional and purposeful movement demonstrates motility. The nutrient broth was supplemented with 0.2% agar, dispensed into test tubes, and sterilized by autoclaving at 121 °C and 15 psi for 15 min. The inoculated test tubes were incubated for 24 hours. Diffused growth, which spreads throughout the medium, indicates motility. Non-motile organisms grew along the line of inoculation (Cheesbrough, 2006).

Methyl red: Voges Proskauer test

The separate test tubes containing MR-VP broth were inoculated with the isolates and incubated at 37 ºC for 24–48 h. After incubation, approximately 1/3rd of each culture was transferred into empty glass test tubes and set aside for the MR-VP test. Five drops of the methyl red indicator were added to the remaining broths in the original test tubes, and color change was observed. 12 drops of VP reagent 1 (5% α-naphthol solution in absolute ethanol) were added to each of the broths that were set aside for the VP test. Each culture was shaken to mix the reagent with the rest of the broths. Immediately, 2–3 drops of VP reagent 2 (40% KOH in distilled water) were added, and the cultures were shaken again. The cultures were shaken again every 3–4 min until approximately 15 min had passed. Results were compared with the control (Cheesbrough, 2006).

Citrate test

Willey et al. (2008) stated that the test was used to determine organisms that could utilize citrate as a sole-carbon source for metabolism. Simon’s citrate agar slants were prepared according to the manufacturer’s instructions. The slants were inoculated by streaking over the surface with a loopful of an 18-hour-old culture and incubated at 37 °C for 48 hr. Growth on agar indicated a positive result, while a change in color from green to blue and the absence of color change indicated a negative change.

Urease test

As stated in Cheesbrough (2006), the test determines the abilities of organisms to produce the urease enzyme, which breaks down urea (by hydrolysis) to give ammonia and CO2. Test organisms were inoculated in test tubes containing 3 mL of sterile Christensen’s modified urea broth and incubated at 35–37 °C for 24 hr. The pink color in the medium indicates a positive result.

Starch hydrolysis test

To determine the isolate's abilities to hydrolyze starch, 50 µL of liquid cultures of each isolate were dropped on a starch-based solid medium containing, per liter, 3 g meat extract, 10 g starch, and 15 g agar (Cheesbrough, 2006).

Gelatin liquefaction test

Willey et al. (2008) stated that gelatin agar medium was composed of 120 g/L of gelatin, 30 g/L of sodium chloride, 5 g/L of peptone, 3 g/L beef extract, 15 g/L agar, and 100 mL of distilled water. A small inoculum of the isolates was stabbed about three-quarters of the way to the bottom of a tube of deep agar with the inoculating needle. The separate stab tubes for each of the isolates were incubated at 37 ºC for 24–48 hr. The incubated stab and the un-inoculated control tubes were placed into the refrigerator for approximately 30 min. The inoculated stab tubes were compared with the control by tapping the tubes gently.

Nitrate reduction test

As stated in Cheesbrough (2006), the test determines the ability of microorganisms to reduce nitrate to nitrite using a reductase enzyme. Nitrate broth was prepared by dissolving 30 g of sodium chloride (NaCl), 3.0 g meat extract, 5.0 g peptone, and 1.0 g potassium nitrate (KNO3) in 1000 mL of water. The broth was inoculated with the isolates in sterile test tubes and incubated for 48 hours. Nitrate reagents I and II were added to the test samples after incubation; a deep red coloration within 5 min is nitrate reductase positive. If no color change is observed, the result is not conclusive. Then a small amount of zinc is added to the broth. If the solution remains colorless, nitrate and nitrite reductase are present. If the solution turns red, nitrite reductase is not present.

Hydrogen sulfide production test

Willey et al. (2008) stated that the test determines the organism's ability to reduce sulfur compounds. Triple sugar iron agar slants were prepared, and each isolate was inoculated into test tubes by streaking the inocula across the top of the slants and stabbing the slant tubes to the bottom. Tubes were incubated at 28 ºC for 24 hr. A positive result is indicated by the formation of black color coupled with displacement of the agar slant and red to yellow color observation.

Sugar fermentation test

Willey et al. (2008) stated that the test determines the abilities of the isolates to ferment glucose, sucrose, lactose, mannitol, maltose, xylose, arabinose, and fructose, as well as their ability to produce gas. The fermentation medium contained 1% peptone water and 5 drops of 0.2 % bromothymol blue indicator solution. Then, 9 mL of medium was dispensed into clean dry test tubes in which Durham tubes had been dropped (inverted and without air space) and sterilized by autoclaving at 121 ºC and 15 psi for 15 min. One milliliter of the sterile 5% test sugar solution was added to the medium inoculated with a loopful of the test organisms and incubated at 30 ºC for 24 hr. A change in color of the medium (from blue to yellow) was recorded as a positive reaction, while the presence of gas in Durham tubes indicates gas production.

Oxidase test

Willey et al. (2008) stated that the test identifies any organism that produces the enzyme oxidase. A loopful of isolates was transferred into pieces of Whatman No. 1 filter paper, impregnated with a freshly prepared oxidase test reagent (N, N, N`, N` tetra-methyl-phenylene diamine), and smeared. Oxidation of the phenylene diamine in the reagent to a dark purple or blue color within 10 seconds indicates a positive result.

Casein hydrolysis test

The casein hydrolysis was observed by observing zones of clearing after 24 h of incubation. For this purpose, 50 µL liquid cultures of each isolate were dropped on a casein-based solid medium containing (per liter) 10 g casein and 15 g agar. The inhibition zones were determined after 24 hours (Cheesbrough, 2006).

Molecular identification

Genomic DNA extraction

The bacterial/yeast stain with the highest growth in diameter and most degradative was selected and characterized molecularly. In this method, 100 µL of blood sample was added to 200 µL of biofluid/cell buffer and 20 µL proteinase K and were mixed thoroughly and incubated at 550 C for 10 mins. Then, 420 uL of genomic binding was added to the resultant mixture. The mixture was transferred to the Zymo-Spin TM IIC-XL Column in a collection tube and was centrifuged at 12,000 xg for 1 min, and the flow through was discarded. Also, 400 µL of DNA pre-washed buffer was added to the spin column in a new collection tube and centrifuged for 1 min. Thereafter, 700 µL of g-DNA wash buffer was added to the spin column and centrifuged for 1 min, and the flow through was discarded. The collection tube with the flow through was discarded. Moreover, 50 µL DNA elution buffer was added to the spin in a clean collection tube, incubated for 5 min, and centrifuged for 1 min to elute the genomic DNA.

Agarose gel electrophoresis

Two percent (2%) agarose gel was prepared by dissolving 1.2 g of agarose in 60 mL of 1X TAE buffer. The mixture was heated to a clear solution using a microwave oven and cooled to about 50 °C. About 3 µL of ethidium bromide was thoroughly mixed with the solution. The agarose preparation was carefully poured into a gel tray with the gel comb in place and allowed to solidify. The tray was loaded into the gel tank, and 1X TAE buffer was poured into the tank, ensuring the gel was properly submerged. The gel comb was carefully removed, and 5µL of DNA was mixed with 2µL of loading dye and loaded into the holes. The tank was connected to the power pack and set to run at 100 volts for 20 min. The bands were viewed using the gel documentation system (VILBER, GERMANY) (Uba, 2019).

Polymerase chain reaction (PCR) protocol

The PCR products were cleaned using Exo/SAP. Exo/SAP Master mix was prepared by adding 50.0 µl Exonuclease I (NEB M0293) 20 U/ul and 200.0 µl Shrimp Alkaline Phosphatase (NEB M0371) 1 U/ul in a 0.6 ml microcentrifuge tube. Exo/SAP Mix of 2.5 µl was then added to 10.0 µl of PCR Mixture, mixed well, and incubated at 37 °C for 30 minutes, after which the reaction was stopped by heating at 95 °C for 5 minutes. Purification was done with the ABI V3.1 Big Dye Kit according to the manufacturer's instructions. The labeled products were then cleaned with the Zymo Seq clean-up kit. A sequencing binding buffer of 240 µl was added to the 20 µl sequencing reaction, which was then transferred to a Zymo-SpinTM IB-96 plate mounted onto a collection plate. The mixture was centrifuged at 3,000 x g for 2 minutes. A sequencing wash buffer of 300 µl was added to each well of the plate and centrifuged at 3,000 x g for 5 minutes. 15 µl of water was directly added to the column matrix of the filter plate. The ZymoSpin™ IB-96 plate was placed on top of the supplied 96-Well PCR plate and mounted onto the collection plate, which was then centrifuged at 3,000 x g for 2 minutes to elute the DNA (Uba, 2019).

Sequencing protocol

The cleaned PCR products were sent for sequencing, which was conducted using the automated DNA sequencer (Perkin-Elmer) in line with the sequencing kit protocol at the Forestry and Agricultural Biotechnology Institute (FABI) Sequencing Facility, University of Pretoria, South Africa (Uba, 2019). Sequence data generated were analyzed with Geneious version 9.0.5.

Blasting and phylogenetic analysis

Revising the sequences of the 16S rRNA genes obtained using BioEdit software, corrected sequences duplicated in a FASTA format, and completed on the National Centre for Biotechnology Information (NCBI) website, The blasting of DNA sequences was performed. Homologies of the gene sequences were checked and compared with the sequences of the NCBI database aligned using MAFFT software (Uba, 2019). The evolutionary history was inferred using the Maximum Likelihood method and the Tamura-Nei model (Tamura et al., 2021). The tree with the highest log likelihood (-38.67) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of estimated pairwise distances using the Tamura-Nei model and selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 13 nucleotide sequences. There was a total of 28 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

3.0 Results

The results of the colony diameter, clearance zone, and enzymatic activity indices of the Screened isolates are presented in Table 1. Among the thirteen (13) isolates that were screened, namely RB1, RB2, RB3, PSB1a, PSB1b, PSB1c, PSB1d, PSB2a, PSB2b, PSB2c, Y1, Y2, Y3; Y3 had the highest enzymatic activity index of 3.79 units, followed by strains RB1 and PSB1b with the enzymatic activity indices of 2.75 and 2.74 units, respectively, and thus selected for characterization and composting inocula.

Table 1: Amylase activity of the microbial isolates

Isolated code	Colony diameter (mm)	Clearance diameter (mm)	Amylase activity index
RB1	13.00 ±0.47	0	1.00
RB2	0	0	0
RB3	10. 00 ±0.82	0	1.00
PSB1a	0	0	0
PSB1b	0	0	0
PSB1c	11.00 ±1.41	0	1
PSB1d	0		0
PSB2a	21.30 ±2.90	28.30 ±2.50	2.33
PSB2b	0	0	0
PSB2c	0	0	0
Y1	0	0	0
Y2	15.70 ±0.47	0	1
Y3	11.00 ±2.16	13.00 ±2.16	2.18

Characterization and Identification of the Selected Microbial Isolates

The result of the microscopic and biochemical properties of the selected isolates is presented in **Table 2**. From the microscopic results, PSB2c and Y3 were Gram-positive, while RB1 produced a negative result for Gram stain. For the shape under the microscope, most isolated strains have rod shape. Also, most isolates were positive for catalase, motility, citrate, methyl red, Voges Proskauer, maltose, rhamnose, fructose, and glucose. Some were negative for the hydrogen sulfide test, nitrate reduction, urease, gelatin, and xylose. Table 2 represents the blast profile of 16S rRNA gene sequences from the National Centre for Biotechnology Information (NCBI). The result showed that *Bacillus thuringensis* was the most blasted (98.49%), followed by *Candida tropicalis* (94.83%), and Enterobacter sp. (94.06%) using NCBI BLAST software, the phylogenetic relationship depicting the relatedness of Enterobacter sp. RB1 (KX548942.1), *Bacillus thuringensis* PSB2c (ON939710.1), and Candida tropicalis Y3 (KY112741.1) to similar bacteria is shown in Figure 1. From the results, *Bacillus thuringensis* PSB2c and Candida tropicalis Y3 had similar ancestry, while Enterobacter sp. RB1 deviated in ancestry.

Table 2: Microscopic and biochemical profile of the selected isolates

Isolate code/ Test	RB1	PSB2c	Y3
Gram reaction	-	+	+
Shape	Rod	Rod	ND
Catalase	+	+	+
Indole	+	+	-
Oxidase	-	+	+
Motility	+	+	+
Citrate	+	+	-
H_2S production	-	-	-
Methyl red	+	+	+
Voges Proskauer	+	+	-
NO_3 reduction	-	-	+
Urease	-	-	-
Gelatin	-	-	-
Sucrose	+	+	-
Glucose	+	+	-
Lactose	+	+	-
Maltose	+	+	-
Arabinose	-	+	-
Inositol	-	+	-
Rhamnose	+	+	+
Fructose	+	+	+
Galactose	+	+	-
Xylose	-	-	-

Key: (+) represents positive, (-) represent negative

Table 3: Blast profile of 16S rRNA gene sequences from the National Centre for Biotechnology Information (NCBI)

Isolate code	Mole - cular ID NO	Description	Scientific name	Max score	Total score	Query Cover (%)	E Value	% ID	Accession length	Access - on NO from GenBank
RB1	4_907-R_B07_04.ab1	*Enterobacter* sp. strain BAB-5981 16S ribosomal RNA gene, partial sequence	*Enterobacter* sp.	1214	1214	99	0.0	93.06	1401	KX548942.1
PSB2c	6_785F_C11_08.ab1	*Bacillus thuringiensis* strain D456 16S ribosomal RNA gene, partial sequence	*Bacillus thuringiensis*	1144	1144	100	0.0	98.49	1422	ON939710.1
Y3	8_ITS1_F10_16.ab1	*Candida tropicalis* isolates S75AF internal transcribed spacer 1, partial sequence	*Candida tropicalis*	797	797	100	0.0	94.83	503	KY112741.1

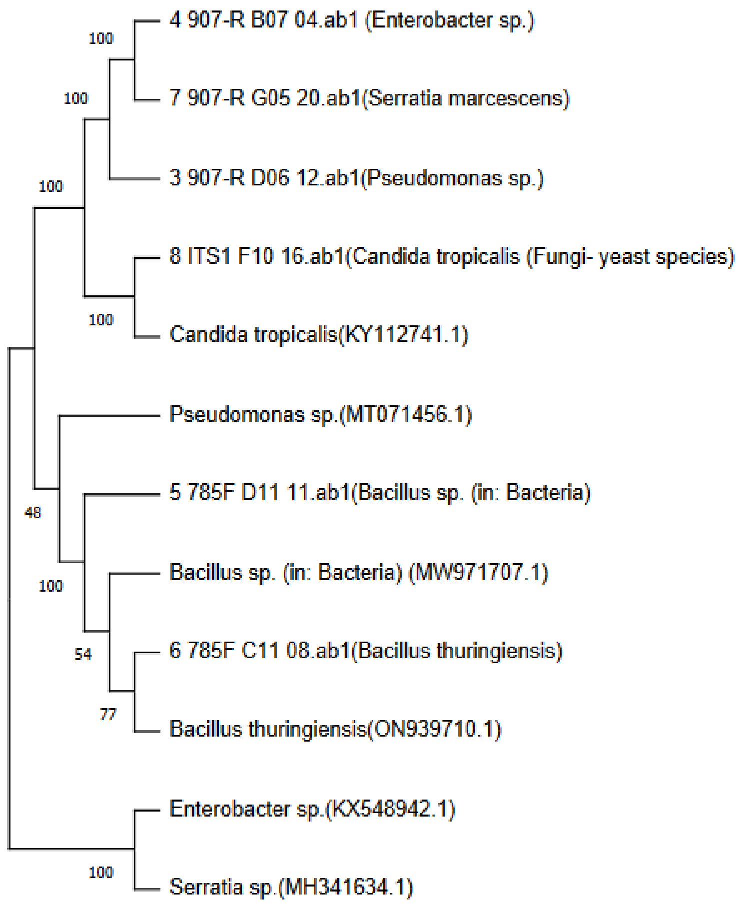

Figure 1: Phylogenetic relationship depicting the relatedness of *Enterobacter* sp. RB1(KX548942.1), *Bacillus thuringensis* PSB2c (ON939710.1) and *Candida tropicalis* Y3 (KY112741.1) to similar bacteria

4.0 Discussion

Beneficial microorganisms capable of degrading solid wastes in the environment have not been fully optimized due to limited characterization. The beneficial microbes for solid waste degradation in this study were isolated and characterized. The results revealed that three strains, namely RB1, PSB2c, and Y3, out of the thirteen beneficial microbial strains, demonstrated dormant amylase, protease, cellulose, ligninase, and lipase enzymatic catalytic activities on different substrates and were finally selected as effective microorganisms or microbial consortiums for this study (Pushpa et al., 2016).

In this study, the selected three effective microbial strains were characterized biochemically and molecularly, and the results revealed the strains were Enterobacter sp. RB1 (KX548942.1), Bacillus thuringensis PSB2c (ON939710.1), and Candida tropicalis Y3 (KY112741.1), although Bacillus thuringensis PSB2c (ON939710.1) and Candida tropicalis Y3 (KY112741.1) showed common ancestry as depicted in the phylogenetic tree. The identified microbial strains have been implicated in the decomposition of both organic and inorganic waste materials by several researchers (Ogbo and Okonkwo, 2012; Ab Muttalib et al., 2016; Pushpa et al., 2016; Jahangir et al., 2019) and are members of the species included in effective microbes (EM) formulation (Saad et al., 2013; Aslanzadeh et al., 2019). Ogbo and Okonkwo (2012) reported that Enterobacter sp. possession of flagella motility, production of cellulose and pectinase enzymes, as well as the ability to utilize a wide range of sugars, could have contributed to the ability of this isolate to colonize the roots of tested seedlings. Zhao et al. (2017) reported that Bacillus species can interact with each other and form a stable micro-ecology system to improve the degradation rate of organic matter, increasing the maturity of kitchen wastes and reducing the toxin products of the reaction. It can produce amylase and specifically degrade starch to small-molecule carbohydrates. It is a benign, ambient plant root-colonizing bacteria. *B. thuringensis* has been developed as a biofertilizer and biocontrol agent via genomics and genetic engineering techniques.

Conclusion

The study has shown that beneficial microorganisms that degrade solid wastes can be isolated and characterized using biochemical and molecular methods, where strains of the biodegrade were revealed as *Enterobacter sp.* RB1 (KX548942.1), *Bacillus thuringensis* PSB2c (ON939710.1), and *Candida tropicalis* Y3 (KY112741.1), although *Bacillus thuringensis* PSB2c (ON939710.1) and *Candida tropicalis* Y3 (KY112741.1) had similar ancestry in phylogenetic relatedness. Therefore, the characterization of these beneficial microorganisms has provided essential information on solid waste management strategy.

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