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Assessing the Nutrient Quality of Biofertilizer Produced from Organic Waste Using Lysinibacillus Macroides and Alcaligens Faecalis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Biofertilizers are ecofriendly fertilizers that are produced via degradation of wastes by microorganisms. The efficiency of Lynsibacillus macroides and Alcaligens faecalis in the production of fertilizer from organic wastes was evaluated. The bacterial isolates were isolated from soil samples collected from fallow patch of land in the Rivers State University farm using standard microbiological methods. The test bacteria were identified by conventional and molecular techniques. Organic wastes including cassava peels, elephant grass and poultry droppings used in this study were collected from the Rivers State University Farm. Three experimental treatments were used in this study; treatment 1 contains 300g of the composite substrate without any organisms and served as control, treatment 2 contains 300g of the composite substrate and 200ml of Lynsibacillus macroides while treatment 3 contains 300g of the composite substrate and 200ml of Alcaligenes faecalis. The treatments were allowed to degrade for 10 days. The pH, temperature, nitrogen, phosphorus, potassium and total organic carbon were determined using standard analytical method. Means of physicochemical parameters in treatment 1, 2 and 3, respectively were: pH 8.3±0.7, 8.6±0.4 and 9.0±0.3; Nitrogen: 2.63±0.08, 1.97±0.03 and 1.51±0.01; phosphorus: 4.71±0.01, 4.43±0.01 and 3.52±0.02; potassium: 604.10±2.12, 591.00±1.41421 and

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504.20±2.83; total organic carbon: 31.75±0.78, 23.04±0.04 and 17.56±0.01 mg/kg. The treatment which was supplemented with Lysinbacillus macroides produced more nitrogen, phosphorus, potassium and total organic carbon than treatment which was supplemented with A. faecalis. Statistical analysis showed that there was no significant difference (P≤ 0.05) between the quantity of nitrogen produced by L. macroides and A. faecalis. Although the amount of nitrogen produced by both organisms were significantly different from the control. More so, statistical analysis showed that the quantity of phosphorus, potassium and total organic carbon produced by L. macroides was significantly higher (P≤ 0.05) than those produced by A. faecalis and the control. Thus, Lynsibacillus macroides is a better biofertilizer producer than A. faecalis.

Keywords: Biofertilizer; organic waste; Alcaligens faecalis; Lysinibacillus macroides; nutrient quality.

1. INTRODUCTION

Conventional agriculture plays an important role in meeting the food needs of a growing human population, which has led to an increasing dependence on the use of chemical fertilizers and pesticides for increased productivity [1,2] Chemical fertilizers are industrially made substances which are composed of known quantities of nitrogen, phosphorus and potassium. The use of chemical fertilizers causes air and ground water pollution as a result of eutrophication of water bodies [3] According to Chun-Li et al. [4] though the practice of using chemical fertilizers and pesticides accelerates soil acidification, it also poses the risk of contaminating ground water and the atmosphere. It also weakens the roots of plants thereby making them to be susceptible to unwanted diseases. In this regard, attempts have recently been made towards the production of nutrient rich high-quality fertilizer (Biofertilizer) to ensure bio-safety and productivity. Biofertilizer has been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. These potential biological fertilizers would play the key role in productivity and sustainability of soil and also protect the environment as eco-friendly and cost-effective inputs for the farmers [5] Organic farming is one of such strategies that not only ensures food safety but also adds to biodiversity of soil [6] the application of microbial degraded organic waste (biofertilizer) to the soil increases the microbial biodiversity which constitutes all kinds of useful bacteria and fungi including the arbuscular mycorrhizal fungi (AMF) called plant growth promoting rhizobacteria (PGPR) and nitrogen fixers. There are so many microorganisms thriving in the soil, especially in the rhizosphere of plant. A considerable number of these microorganisms possess a functional relationship and constitute a holistic system with plants. They have beneficial effects on plant growth [7].

Biofertilizers keep the soil environment rich in all kinds of macro and micro nutrients via nitrogen fixation, phosphate and potassium solubilisation or mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil [8] Biofertilizers, when applied as seed or soil inoculant participate in nutrient cycling and leads to increase crop productivity. Generally, 60 to 90% of the total applied fertilizer is lost and the remaining 10 - 40% is taken up by plants. Hence biofertilizers can be important component of integrated nutrient management systems for sustaining agricultural productivity and a healthy environment [9] Biofertilizers are products containing living cells of different microorganisms which have ability to convert nutritionally important elements from unavailable to available form through biological processes [7] There is an increased level of wastes dump sites in Port Harcourt which produce unpleasant odour and contribute to transmission of diseases. Turning the wastes to biofertilizers would boost increase crop yield. Despite reports from previous studies about the use of microbial cells in the production of eco-friendly fertilizers there is still dearth of information in the microbial cells that yields, through biodegradation, faster and higher nutrients required by plants for growth. Thus, this study was aimed at evaluating the biofertilizer produced by Lyncibacillus macroides and Alcaligens faecalis.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The study location was Rivers State University Farm, Port Harcourt Rivers state, Nigeria within the coordinates $04^{0}48.2188^{,}$' N and 006058 .6151, E. The University is located in Port Harcourt metropolis which is located in the Niger Delta region of Southern Nigeria. The city is situated between latitudes $04^{0}48.2188^{,}$' N and

longitude $006^0$58 .6151'' E, approximately 50km from the Atlantic coast. Precipitation averages 3,030mm annual and a temperature average of 23°C. The main occupation of the people is farming and fishing.

2.2 Collection of Samples

2.2.1 Soil Sample

Soil samples used in this study were collected from the Rivers State University Farm. The soil samples were collected into sterile black polythene bag with the aid of a clean auger and were transported to the Microbiology Research Laboratory, Department of Microbiology, Rivers State University for analysis.

2.2.2 Organic Waste Samples

Organic wastes including cassava peels, elephant grass and poultry droppings used in this study were collected from the Rivers State University Farm. These samples were collected into black polythene bag and transported to the Microbiology Research Laboratory, Department of Microbiology, Rivers State University for treatment.

2.3 Isolation of the test Microorganisms

The bacterial isolates, Lysinibacillus macroides and Alcalegenes feacalis used in this study were isolated using serial dilution and standard plate count [10] In this method, 10g of the soil sample was transferred into a 250ml conical flask containing sterile ninety millilitre (90ml) of normal saline and was serially diluted to obtain dilutions up to 10^{-6}. An aliquot (0.1ml) from the 10^{-3} and 10^{-4} dilutions were inoculated separately in duplicate on prepared Cetrimide agar and Nutrient agar plates respectively. The plates were spread evenly using flamed bent glass rod and were incubated for 24h at 37^0C. The colonies that grew on the plates were subcultured on freshly prepared nutrient agar in other to obtain pure cultures. Pure cultures were further characterized using conventional and molecular methods. The pure isolates were transferred into well labelled bijou bottles containing 5ml of sterile 10% (v/v) glycerol which were stored frozen in a deep freezer for further use.

2.4 Identification of the test Bacterial Isolates

Pure isolates were characterized and identified based on their colonial, morphological, biochemical and molecular identification methods [11] The colonial identification includes size, colour, shape, elevation and texture. The morphological includes gram stain and motility. The biochemical includes sugar fermentation tests, oxidase test, Methyl Red-Voges Proskauer test, citrate test, catalase test and indole test. The tests were all carried out using standard methods [11].

2.5 Molecular Based Technique

The molecular method was used in characterizing the bacterial isolates [12] In this method, bacterial DNA was extracted using the boiling method. This was done by transferring 24 hours old culture of test isolate into Luria Bertani (LB) medium and re-incubated for 24 hours. After incubation, 5ml of the broth culture was spun at 14000 rpm for 3 minutes. The cells were later resuspended in 500 µl of normal saline and heated at 95°C for 20 minutes. The heated bacterial suspension was cooled on ice and spun for 3 minutes at 14000 rpm. The supernatant containing the DNA was transferred in to a 1.5 ml micro centrifuge tube and stored at -20°C. The bacterial DNA was quantified using the Nanodrop 1000 spectrophotometer. The 16S rRNA was amplified using the 27F and 1492R primers on ABI 9700 Applied Biosystems thermal cycler in a total volume of 25µl for 35. The PCR mix used was composed of the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl). The forward and reverse primers at a concentration of 0.4M and the extracted DNA represented the template. The PCR conditions were set: initial denaturation was 95ºC for 5 minutes; Denaturation was 95ºC for 30 seconds; annealing was 52ºC for 30 seconds; extension was 72ºC for 30 seconds for 35 cycles and the final extension was 72ºC for 5 minutes. The product was later resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a UV transilluminator. The BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa was used in sequencing. Phylogenic analysis was carried out by editing resulting sequences with the aid of the bioinformatics algorithm Trace edit tool having downloaded similar sequences from the National Center for Biotechnology Information (NCBI) data base using BLASTN. Downloaded sequences were aligned using ClustalX and the evolutionary history was inferred using the Neighbor-Joining method in MEGA 7.0 [13] The bootstrap consensus tree inferred from 500 replicates [14] was taken to represent the

evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method [15]

2.6 Preparation of Substrates of Organic Waste

The organic wastes such as cassava peel, Elephant grass and poultry dropping were sun dried, milled to fine particle and mixed together to form a composite substrate used in this study. Three hundred grams of the composite substrate (100g of each organic wastes) were weighed in three places for the 3 treatments into a foil and further sterilized in an autoclave at 121psi for 15 minutes. This was allowed to cool before they were transferred into the respective reactors (Transparent Plastic containers).

2.7 Experimental Treatments

Three experimental treatments were used in this study for each crop which include,

Treatment 1: which contains 300g of the composite substrate without any organisms,
Treatment 2: which contains 300g of the composite substrate and 200ml of Lynsibacillus macroides
Treatment 3: which contains 300g of the composite substrate and 200ml of Alcaligenes feacalis.

All three treatments were allowed for 10 days and the physicochemical parameters including nutrients were monitored.

2.8 Analysis of Physiochemical Parameters of the Set Ups

The Physicochemical parameters such as pH, temperature, nitrogen, phosphorus and total organic carbon of the respective organic wastes were determined before and after microbial degradation.

2.8.1 Determination of temperature and pH of the treatments

The temperature and pH of the respective treatments were determined using Hanna multiple meters at a constant interval of 48 hours all through the period of degradation of the organic wastes in the three treatments in order to optimize the treatment to the optimal condition.

2.8.2 Determination of phosphorus

A 5 ml aliquot of the filtrate was taken into a 25 ml volumetric flask. Five millilitres of ammonium vanadate solution and 2 ml stannous chloride solution were added. The volume was made up to 25 ml with distilled water and allowed to stand for 15 minutes for full colour development. A standard curve was developed concurrently with phosphorus concentrations ranging from 0, 5, 10, 15 to 20 mg/kg organic material. The absorbance of the sample and standard solutions was read on a spectronic 21D spectrophotometer at a wavelength of 470 nm. The absorbance values of the standard solutions were plotted against their respective concentrations to obtain a standard curve from which phosphorus concentrations of the samples were determined.

2.8.3 Determination of nitrogen

Total N was determined by the Kjeldahl method in which poultry manure and plant material were each oxidized by sulphuric acid and hydrogen peroxide with selenium as catalyst. Twenty grams oven-dried sample was ground in a stainless-steel hammer mill and passed through 1 mm sieve. A 0.5 g sample was digested in 10 ml concentrated sulphuric acid with selenium mixture as catalyst. The resulting clear digest was transferred into 100 ml conical flask and made to volume with distilled water. A 5 ml aliquot of the sample and a blank were pipetted into the Kjeldahl distillation apparatus separately and 10 ml of 40 % NaOH solution was added followed by distillation. The evolved ammonia gas was trapped in a 25 ml of 2 % boric acid. The distillate was titrated with 0.1 M HCl with bromocresol green-methyl red as indicator.

Calculation:

$$\% \text{ N/DM} = \frac{(a - b) \text{ X M x } 1.4 \text{ x mcf}}{W}$$

where : a = ml HCl used for sample titration
b = ml HCl used for blank titration
M = molarity of HCl
1.4 = 14 × 0.001 × 100 % (14 = atomic weight of N)
DM = dry matter
w = weight of sample

2.8.4 Determination of organic carbon

Organic carbon content of the respective treatments was determined using the dichromate-acid oxidation method. Ten millilitres (10 ml) each of concentrated sulphuric acid, 0.5 N potassium dichromate solution and concentrated orthophosphoric acid were added

to 0.05 g of sample in Erlenmeyer flask. The solution was allowed to stand for 30 minutes after addition of distilled water. It was then back titrated with 0.5 N ferrous sulphate solution with diphenylamine indicator.The organic carbon content was calculated from the equation:

$$\% \text{ carbon} = \frac{N \times (a-b) \times 3 \times 100^{-3} \times 100 \times 1.3}{W}$$

where: N = normality of ferrous sulphate
a = ml ferrous sulphate solution required for sample titration
b = ml ferrous sulphate solution required for blank titration
w = weight of oven- dried sample in gram
3 = equivalent weight of carbon
1.3= compensation factor allowing for incomplete combustion

3. RESULTS AND DISCUSSION

3.1 Bacterial Organisms Isolate

The biochemical and molecular characterization of the bacterial isolates showed that they were Lysinbacillus macroides and Alcaligenes faecalis. The phylogenic tree of the two bacterial isolates is presented in Fig 1. The result showed that the bacterial isolate with code A2 which was identified phenotypically identified as Bacillus sp has a 100% similarity with Lysinbacillus macroides, while the isolate A3 which was phenotypically identified as Pseudomonas sp has a 100% relatedness with Alcaligenes faecalis.

3.2 Physicochemical Parameters of Degraded Organic Waste

Results of analysis of pH is presented in Table 1. The pH values in all the treatments across the days tended towards alkaline range. The pH of the control, substrate + Lysinbacillus and Alcaligenes faecalis in day one were 9.4, 9.4 and 9.6, respectively. While the pH in day 4 were 8.6, 8.9 and 9.0 for the control, substrate + Lysinbacillus and substrate + Alcaligenes faecalis, respectively. The pH of the control, substrate + Lysinbacillus and substrate + Alcaligenes faecalis in day 6 were 7.9, 8.1 and 9.0. The pH of the control, substrate + Lysinbacillus and substrate + Alcaligenes faecalis in day 8 was 7.3, 8.6 and 8.9, while the pH in day 10 were 8.4, 8.4 and 8.6 for the control, substrate + Lysinbacillus and substrate + Alcaligenes faecalis. The pH values of the control decreased from 9.4 from day 1 to 7.3 in day 8 and increased from 7.3 in day 8 to 8.4 in day 10. The pH of treatment 2 (substrate + Lysinbacillus) decreased from 9.4 in day 1 to 8.1 in day 6 and increased from this value to 8.6 in day 8 and later decreased again to 8.4 in day 10. Similar decrease in pH was observed in treatment 3 (substrate + A. faecalis) as the pH decreased from 9.6 in day 1 to 8.6 in day 10 despite having a constant pH in days 4 and 6.

The result of the temperature is presented in Table 2. The result showed that the temperature for the control increased from 31^0C in day 1 to 39^0C in day 8 and later decreased to 32^0C in day 10. Similar trend in temperature fluctuation was observed in treatments 2 and 3. For treatment 2, the temperature increased from 32^0C in day 1 to 38^0C in day 8 and decreased to 29^0C in day 10. It was also observed that the temperature was constant from day 4 to day 8. While in treatment 3, the temperature increased from 32^0C in day 1 to 42^0C in day 8 but decreased to 34^0C in day 10.

The activities of bacteria in the degradation of the organic waste revealed that there was continuous reduction in the organic wastes especially those with treatment. The result is presented in Table 3. The result showed that the control which had weighed 300g in day one maintained this same weight until day 6 but decreased in weight to 295g and 282g in day 8 and 10 respectively. Treatment 2 which had initial weight of 320g in day 1 decreased in weight continuously to 300g, 250g, 199g and 145g for days 4, 6, 8 and 10 respectively. Similarly, treatment 3 decreased from 320g in day 1 to 300g, 250g, 198g and 145g for days 4, 6, 8 and 10 respectively.

The result of the mineral composition of the experimental treatments is presented in Table 4. The result showed that values of nitrogen, phosphorus, potassium and total organic carbon of the control (treatment1) were 2.697, 4.72, 6,051.6 andc31.2 mg/kg. Nitrogen, phosphorus, potassium and total organic carbon values for treatment 2 were 1.989, 4.42, 5,930.0 and 23.01mg/kg, respectively. While the values of nitrogen, phosphorus, potassium and total organic carbon for treatment 3 were 1.517, 3.53, 5,029.2 and 17.55 mg/kg respectively.

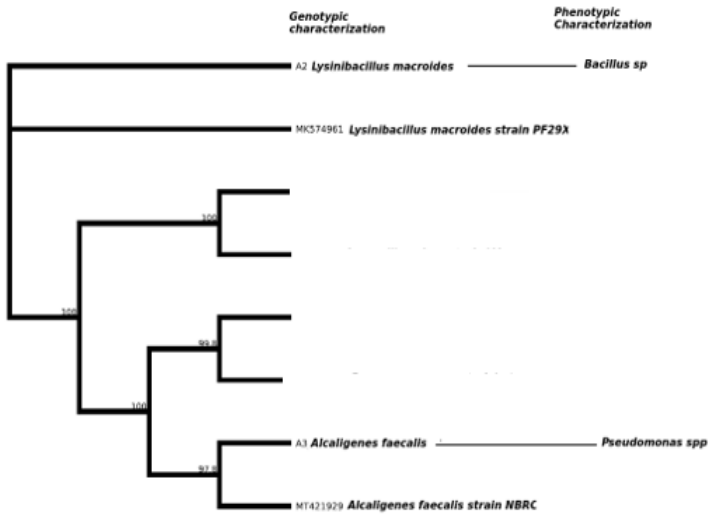

Fig. 1. Phylogenetic tree showing the evolutionary distance between the microbial isolates

Table 1. pH Values of the Microbial Degraded Organic Waste

SET UP	Day 1	Day 4	Day 6	Day 8	Day 10
1	9.4	8.6	7.9	7.3	8.4
2	9.4	8.9	8.1	8.6	8.4
3	9.6	9.0	9.0	8.9	8.6

Table 2. Values of Temperature of the Microbial Degraded Organic Waste

SET UP	Day 1	Day 4	Day 6	Day 8	Day 10
1	31^0C	37^0C	39^0C	39^0C	32^0C
2	33^0C	38^0C	38^0C	38^0C	29^0C
3	32^0C	36^0C	37^0C	42^0C	34^0C

Table 3. Values of Weight of Microbial Degraded Organic Wastes

SET UP	Day 1	Day 4	Day 6	Day 8	Day 10
1	300g	300g	300g	295g	282g
2	320g	300g	250g	199g	145g
3	320g	300g	250g	198g	145g

Table 4. Mineral Composition of the Microbial Degraded ORGANIC waste

Treatments	Nitrogen mg/kg	Phosphorus mg/kg	Potassium mg/kg	TOC mg/kg
1	2.63 ± 0.08^b	4.71 ± 0.01^c	604.10 ± 2.12^c	31.75 ± 0.78^c
2	1.97 ± 0.03^a	4.43 ± 0.01^b	591.00 ± 1.41421^b	23.04 ± 0.04^b
3	1.51 ± 0.01^a	3.52 ± 0.02^a	504.20 ± 2.83^a	17.56 ± 0.01^a

** Means with similar superscript across the column showed no significant difference (P=0.05)*
Key: TOC = Total Organic Carbon

4. DISCUSSION

Microorganisms are known to be widely distributed in different environment and they try to adapt to the environment despite the fluctuations in environmental conditions. However, some which cannot thrive or adapt to the changes die off while those which can adapt

grow and utilize the substrate available as carbon and energy source for their dominance. The biofertilizer production efficiency of L. macroides and A. faecalis on organic substrate was evaluated. The physicochemical parameters such as the pH and temperature of all the treatments fluctuated across the days of composting. The fluctuation could be attributed to microbial activities taking place. Fluctuations in pH and temperature during degradation of organic material has been reported in a previous study [16] The pH values in this study were higher than the pH values of 4.0-5.8 of during composting of rice waste but lower than the pH range of 9.20-10.30 obtained from rice waste+ cow dung+ sawdust composting reported by Oladipupo and Ayorinde Also, the pH values in this current study were higher than the 4.5 reported by [17] There was gradual decrease in pH of the treatments through out the period of experiments. Thus, signifying the activities of microorganisms in degrading the organic wastes and volatilizing organic acids of organic origin including release of ammonia through mineralization of organic nitrogen source. Similar trends in pH value was observed by White et al. who reported that alkaline pH could facilitate the breaking down process of wastes and therefore control pathogenic fungi that prefer acidic growth conditions [18] The pH range at the end of composting which is within the accepted pH range of 6-9 for rapid composting could be attributed to the production of amonnia and nitrogen [19]. The pH is a vital component that affect microbial activities as it plays vital role in metabolism and enzyme function [10] Temperature is a very important factor that is vital for microbial activities as well as other organisms found in the soil. It could influence the enzymatic activity of microorganisms in an environment since temperature ranges above the optimum range could be devasting to enzymatic function [10] The temperatures of all the treatments increased within day four from the initial temperature and decreased rapidly in day ten as the compost stabilized. [20] also reported increased temperature in day two from an initial temperature during composting. The temperature did-not rise to thermophylic phase as reported by several authors (Yang, Saidi et al. [18] This could be due to the proper aeration of the reactor used for composting as well as the site location which was well carved to maintain a humid environment. Excessive aeration have been reported to increase costs and slowing down of composting process through loss of heat Scaglia et al., The highest temperature range which was observed in day 8 of treatment 3 revealed that treatment 3 very high amount of energy than treatments 1 and 2. This loss of energy in treatment 3 could also be the reason while it yielded lesser nitrogen, phosphorus, potassium and total organic carbon due to aeration. Aeration rate have been reported to directly affect the quality of compost product including loss of nitrogen, and energy consumption (Scaglia et al., More so, the findings in this current study revealed that the temperature had an effect in the pH such that an increase in temperature resulted to the reduction in the pH of the treatments. This agreed with [20] who also reported significant decrease in pH during the high-temperature period of composting. The organic wastes were greatly reduced in day 8 and day 10 in all the treatments. the weights of organic wastes in treatments 2 and 3 was drastically reduced in days 8 and 10 compared to treatments 1. This reduction in organic wastes could be attributed to the high rate of microbial activities which is favoured by the temperature and pH. In this current study, the degradation of organic wastes was more efficient in the substrates treated with Lynsibacillus macroides and A. faecalis. However, the degradation of organic wastes between these two microbes showed no significant differece in the quantity of nitrogen produced. The quantity of phosphorus, potassium and total organic carbon produced in treatment 2 was significantly higher than the quntities of similar parameters produced by treatment 3. More so, at the end of the experiment, the nitrogen, phosphorus, potassium and the total organic carbon was more in the treatment 1 (control) than in the other treatments. This could be due to the diverse microorganisms existing in the substrate. Comparatively, the mineral elements produced by Lysinbacillus macroides were higher than the mineral elements produced by A. faecalis.

5. CONCLUSION

In this study, Lysinbacillus macrolides and Alcaligens faecalis were used in producing biofertlizer from organic wastes (cassava peels, elephant grass and poultry droppings). The bacterial isolates produced biofertilizers which could be used in enhancing the fertility of the soil. More so, the nitrogen, phosphorus, potassium and total organic carbon were higher in the biofertilizer produced by Lynsibacillus macrolides than those produced by A. faecalis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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