FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 9 No. 1, January, 2025, pp 218 - 224
DOI: https://doi.org/10.33003/fjs-2025-0901-3038

ISOLATION AND CHARACTERIZATION OF CELLULOLYTIC BACTERIA AND FUNGI FROM CASSAVA WASTE AND MILL SOIL

Majekodunmi Racheal Adedayo and Taofeek Olatunji Lateef

Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, PMB 1530

*Corresponding authors' email: : majekodunmi.adedayo@kwasu.edu.ng Phone: +2348133325627

ABSTRACT

The abundance of cellulose on earth, the need for its biodegradation and the various applications of cellulolytic enzymes in commercial settings have necessitated unrestrained research for novel cellulase producing indigenous microorganisms for local production of the enzyme to meet the ever-growing and enormous demand for it. Soil sample was collected from a cassava processing mill while freshly harvested cassava was peeled and steeped in water for five days. Cellulase degrading bacteria and fungi were isolated from the cassava mill soil and the liquid waste (Cassava steeped water) on nutrient agar and potato dextrose agar using pour plate method under standard laboratory conditions for 48 hours. The isolated bacteria and fungi were identified using cellular morphology and biochemical characteristics; they were screened for cellulolytic ability on carboxyl methyl cellulose media supplemented with 0.5% Congo red and incubated for 48 hours. The bacteria isolated were *Escherichial coli, Pseudomonas* and *Bacillus* species while the fungi isolates were *Aspergillus fumigatus, flavus, terreus, niger*, *Rhizopus* species and *Trichodermal* species. The isolated *Pseudomonas* species has the highest cellulolytic ability of (18.00 mm) in terms of diameter of zone of clearance on the Congo red media among the bacteria, then the *Bacillus* species (15.00 mm) while the least of 1.00 mm was in the *E. coli* isolated. *Aspergillus terreus, fumigatus and niger* were the isolates with highest cellulolytic ability with zones of clearance measuring 15.00, 13.00 and 13.00 mm respectively. Conclusively, the isolated *Pseudomonas*, *Bacillus* and *Aspergillus* species are promising organisms as potential enzyme cellulase producer.

Keywords: Enzyme, Cellulase, Cassava, *Pseudomonas* species, *Aspergillus* species

INTRODUCTION

Recent discoveries on the use of microorganisms as sources of industrially relevant enzymes have led to an increase in the application of microbial enzymes in various industrial processes (Maravi and Kumar, 2020; Suwannaphan *et al*., 2024). The potential of microorganisms as biotechnological sources of industrially relevant enzymes has stimulated interest in exploration of extracellular enzymatic activities in several microorganisms (Buzzini and Martini, 2002). Microbial enzymes are preferred to those from plants and animal sources because they are cheaper to produce and their enzyme contents are more predictable, controllable and reliable (Oyeleke and Oduwole, 2009; Bautista-Cruz *et al*., 2024) and also because of their broad biochemical diversity, feasibility of mass culture and ease of genetic manipulation (Abu *et al.,* 2005).

Enzymes have found several important applications in many industries such as the textile, detergents, food and beverages, paper mill, bioremediation, pharmaceuticals and medicine, and leather tanin industries (Asgher *et al*., 2007; Bautista-Cruz *et al*., 2024).

The discovery of enzyme producing microbes rely on screening a large number of organisms for an enzyme activity with a specific set of biochemical and physical characteristic that suit the targeted applications (Miyoko and Henrik, 2000; Singh *et al*., 2019; Singh *et al*., 2021). Traditionally, microbial screening processes have involved evaluation of enzymes isolated from environments rich in the substrate of interest or rich in microorganisms that have a long history of use in the process of enzyme production (Djoulde, 2004). Several fungi and bacteria have been used and are still being use in the process of enzyme production. Such fungi as *Aspergillus*, *Trichoderma*, *Penicillium*, *Fusarium* and *Rhizopus* have several species that have been used while several species of *Bacillus* and *Pseudomonas* are among bacteria that have been considered for various enzyme productions. The selection of microorganisms is a very critical stage in the screening process and can be quite subjective especially if the numbers of microorganisms involved are large Malik and Javed, 2021; Balla *et al*., 2022).. Cellulase refers to a group of enzymes which, acting together, hydrolyze cellulose (Maravi and Kumar, 2020; Suwannaphan *et al*., 2024). There are numerous applications of cellulases in various industries, including food, brewery and wine, animal feed, pollution treatment, textile and laundry, pulp and paper, agriculture waste management, protoplast production, genetic engineering as well as in research and development (Anish *et al*., 2006; Bautista-Cruz *et al*., 2024). Cellulase production has attracted a worldwide attention due to the possibility of using this enzyme complex for conversion of abundantly available renewable lignocellulosic biomass for the production of carbohydrates for numerous industrial applications (Hayward *et al.,* 2000).

Cassava, (*Manihot esculenta*), is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root, a major source of carbohydrates. Cassava is the third largest source of carbohydrates food in the tropics, after rice and maize (Akinrele *et al.,* 2009). Cassava is one of the most important food crops in tropical developing countries and is consumed by many people as major staple (Akinrele *et al.,* 2009). Cassava starchy storage roots are rich in carbohydrates but lack proteins. In some areas of Africa, it constitutes over 50% of the daily diet of the people (Kobawila *et al.,* 2005). Nigeria is the world's largest producer of cassava (Akinrele *et al.,* 2009).The consumption of cassava and other commercial utilization results in generation of tons of cassava waste in term of solid waste and effluents from the industries and individual usage. The waste is very rich in cellulose and could serve as organic carbon rich substrate for heterotrophic microorganisms to utilize in the

production of cellulase. This will enhance waste management and mitigate the challenge of pollution and the cost of managing solid waste. The abundance organic carbon in the cassava peel and the waste water discharged into the soil make such environment natural repository for fungi and bacteria with cellulolytic ability and could be sought for such (Nwogwugwu *et al*., 2018). Therefore, cassava processing mill soil and effluents can be a source of fungi and bacteria of industrial importance. Screenings for microorganisms with higher cellulase activities could therefore, facilitate the discovery of novel cellulase producing organisms suitable to new industrial applications. The challenges in cellulase production involve identifying high cellulase producers. Discovery of local novel cellulase producers to will go a long way in enhancing production of this industrially important enzymes and in no small measures alleviate the challenge of the cost of importing the enzyme. Thus, the growth and expansion of local cellulase utilizing industries will be strengthened (Ezeagu *et al*., 2023). Previous authors have isolated cellulolytic bacteria and fungi from various agricultural wastes but there is need to explore the environments for more of these microbes to be able to cope with the ever increasing demand for this enzyme and the need to properly managed cassava tones of cassava waste generated in the country annually. This research was therefore aimed at isolation and screening for cellulase producing fungi and bacteria from steeped cassava (*Manihot esculenta*) and soil of cassava processing mill.

MATERIALS AND METHODS

Collection of samples

Some tubers of cassava were uprooted from cassava farm very early in the morning. They were collected in polythene bags and transported to the laboratory immediately for further studies. Soil sample from cassava processing mill was collected in sterile polythene bag at the depth of 5 cm below the surface with the aid of a sterile hand trowel and transported to the laboratory immediately for further studies.

Preparation of samples

The cassava tubers were peeled and then washed to remove dirt. The washed tubers were cut in halves and transferred into clean container. Water was poured to cover the bulbs. The cassava tubers were allowed to remain inside the water for five days. During this five-day period the cassava bulbs underwent fermentation by microorganisms and enzyme action. On the fifth day the water was gently decanted from the cassava tubers into a sterile universal bottle, used to prepare serial dilution.

Isolation of fungi and bacteria from the soil and water samples

The pour plate method was used to isolate fungi from the soil and the water samples from the steeped cassava. One gram of soil and one ml of the water sample was used in ten folds serial dilution repeatedly for five times. One milliliter of each appropriate dilution was pipetted into sterile Petri dishes, thereafter molten Potato Dextrose and Nutrient Agar media were poured and the Petri dishes were gently swirled for even distribution. The PDA was fortified with 4% Streptomycin sulfate to prevent the growth of bacteria. These plates were allowed to set, inverted, and incubated at 30 and 37 ^{o}C for 48 and 24 hours respectively to allow for the growth of fungi and bacteria. Distinct colonies were sub-cultured repeatedly until pure cultures were obtained. The pure cultures were transferred to agar slants for 72 hours, before they were stored in the refrigerator at 4 ^{o}C for further use (Aneja, 1996; Cappuccino and Sherman, 2002).

Identification of bacterial isolates

Pure cultures of bacterial isolates were used for identification studies. Each isolate was identified on the basis of its morphological, biochemical and physiological characteristics. Bacteria were identified based on the taxonomic descriptions given in Bergey's Manual of Determinative Bacteriology.

Gram's Reaction

Gram staining technique was employed to determine the Gram reaction of each of the pure colonies obtained on the streak culture plates (Fawole and Oso, 2004).

Motility Test

Test tubes were labeled with the code on the stock culture. Semi solid media containing peptone was poured into the test tubes and allowed to set. Using a sterile wire loop a colony of bacteria was stabbed into the semi-solid agar media down to the base and incubated at 25°C for 24 hours. The test tubes were examined for a black precipitate along the line of inoculation or stabbing (Fawole and Oso, 2004).

Catalase Test

The isolates were picked with a flamed wire loop and emulsified in a drop of 6% hydrogen peroxide solution on a clean glass slide. The production of gas bubbles after 10 seconds indicated a positive catalase test (Fawole and Oso, 2004).

Indole Test

Test tube containing sterile tryptone water inoculated with a loop full of the isolate from the Petri dishes containing the bacteria culture after which it was incubated for 48 hours at 37^{o}C, after incubation, 0.5ml of Kovac's reagent was added and stirred and allowed to stand for few minutes. If a red ring forms, it indicates organism is positive, but if there is no ring red colors it means that the organism is negative (Fawole and Oso, 2004).

Identification of fungal isolates

Wet preparation of each fungal isolate was made on clean microscope slides. A drop of sterile distilled water was put onto the surface of a microscope slide using a sterile wire loop. The wet loop was dried by flaming until red hot and allowed to cool at room temperature. After cooling, the loop was used to pick a small portion of the fungal colony from the plates and mixed with a drop of water to form a smear on the surface of the microscope slide. Three drops of lactophenol blue solution were deposited on the smear for 2 minutes. A clean cover slip was carefully placed on the smear so that no air bubbles were trapped. Excess stain was drained off. The slides were examined under the microscope for cellular characteristics. Pure cultures of fungal isolates stored on PDA slants at 4^{o}C were used for identification studies. The isolates were identified on the basis of their morphological and cultural characteristics. The taxonomic schemes described by De Hoog *et al*. (2000) were used to identify the fungal isolates.

Screening for cellulase producing ability of the isolated microorganisms

Screening was done as described by Gupta *et al*. (2012) and used by Maravi and Kumar (2020); Bahatkar *et al*. (2023).

Carboxymethyl cellulose (CMC) agar was prepared by adding 2 % carboxymethyl cellulose (CMC) (w/v) into Potato Dextrose agar as carbon source and supplemented with 4 % streptomycin sulfate. The same process was repeated for Nutrient agar without the addition of streptomycin. Bacterial and fungal isolates were individually inoculated on CMC agar plates and incubated for 1 and 2 days at 37 and 30 ^{0} C for bacteria and fungi respectively. The plates were flooded with 0.5 % Congo red adjusted to pH 7.0-7.2 for 20 minutes and washed with 1 M NaCl for 15 min. The clear zone formed by the isolates indicated their ability to hydrolyse cellulose. The clearance zone was measured with the aid of transparent ruler in millimeters.

RESULTS AND DISCUSSION

Identification of microorganisms from the cassava processing mill soil and steeped water

Results in Tables 1 and 2 show the identities of the microorganisms isolated from steeped cassava and cassava processing mill soil. The microorganisms were identified by comparing their cultural, morphological and biochemical characteristics with those of known taxa.

Frequency of occurrence of the fungal and bacterial species

The frequency of occurrence of each bacterial and fungal species is shown in Table 3. The percentage of the occurrence was also indicated.

Cellulolytic ability of Isolated Bacteria and Fungi

The cellulolytic ability of the isolated microorganisms is presented in Figure 1 and Plate 1. *Pseudomonas* species isolated was the best cellulolytic bacteria with a zone of cellulose hydrolysis of diameter of 15.00 mm while the least diameter of zone of clearance of 1.00 mm was recorded by the *Escherichia coli* isolated. *Aspergillus tereus, fumigatus* and *niger* has the highest cellulolytic ability with zones of clearance of 15.00, 13.00 and 13. 00 mm respectively.

Table 1: Grams reaction and biochemical characteristics of bacterial isolates

Isolates	Shape	Gram	Catalase	Indole	Motility	Pigmentation	Organisms
B1/BSC 1	Rod	negative	positive	Positive	Motile	Cream	*Escherichia coli*
B2/BSC3	Rod	negative	positive	Negative	motile	Green	*Pseudomonas* sp.
B3/BSC 2	Rod	positive	Positive	Negative	Motile	Cream	*Bacillus* sp.

Table 2: Identification of fungal isolates

Isolates	Texture	Surface colour	Reverse colour	Hyphae	Conidiophore/ Sporangiophore	Organisms
F1/FSC4	Powdery	Black	Pale yellow	Septate	Conidiophore	*Aspergillus niger*
F2/FSC3	Powdery	Beige	Pale yellow	Septate	Conidiophore	*Aspergillus terreus*
F3/FSC5	Wooly	Gray green	yellow	Septate	Conidiophore	*Aspergillus fumigatus*
F4/FSC6	Wooly	White with green patches	Pale	Septate	Conidiophore	*Trichoderma* spp
F5/FSC2	Wooly	Olive	Cream	Septate	Conidiophore	*Aspergillus flavus*
F6	powdery	Gray	Yellow	Septate	Conidiophore	*Aspergillus* spp
F7/FSC1	Cotton	Gray	Pale	Aseptate	Sporangiophore	*Rhizopus* spp

Table 3: Percentage occurrence of isolated fungal and bacterial species

Fungal/Bacteria species	Soil	Water from steeped cassava	Percentage (%)
Aspergillus fumigatus	+	+	20
Aspergillus flavus	+	+	20
Rhizopus sp,	+	+	20
Aspergillus terreus	+	+	20
Trichoderma sp.	+	+	20
Aspergillus niger	+	+	20
Aspergillus sp.	+	-	10
Bacillus sp.	+	+	20
Escherichia coli	+	+	20
Pseudomonas sp.	+	+	20

Key: + present
_absent

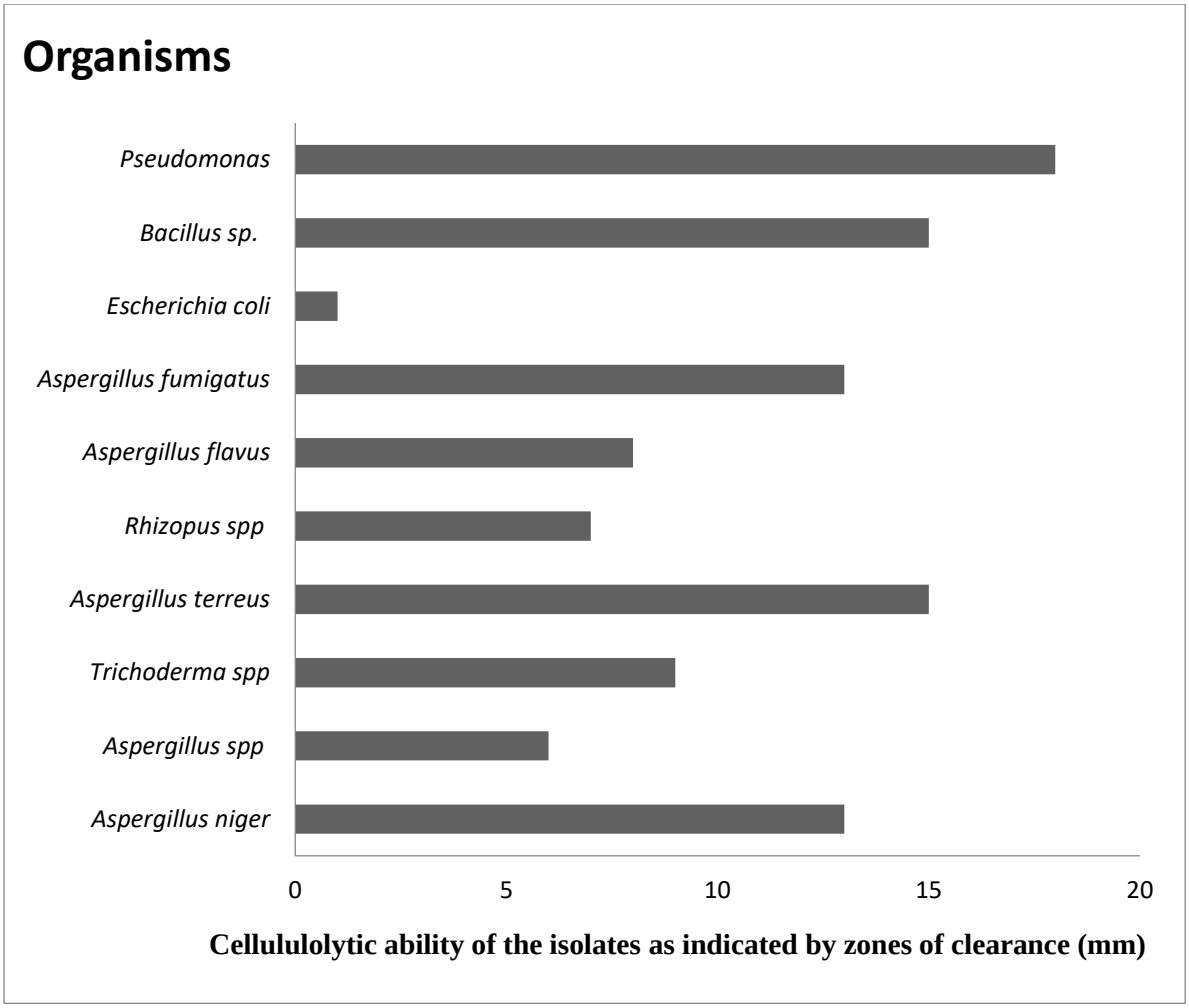

Figure 1: Cellulolytic activity of fungal and bacterial isolates as depicted by zones of clearance (mm)

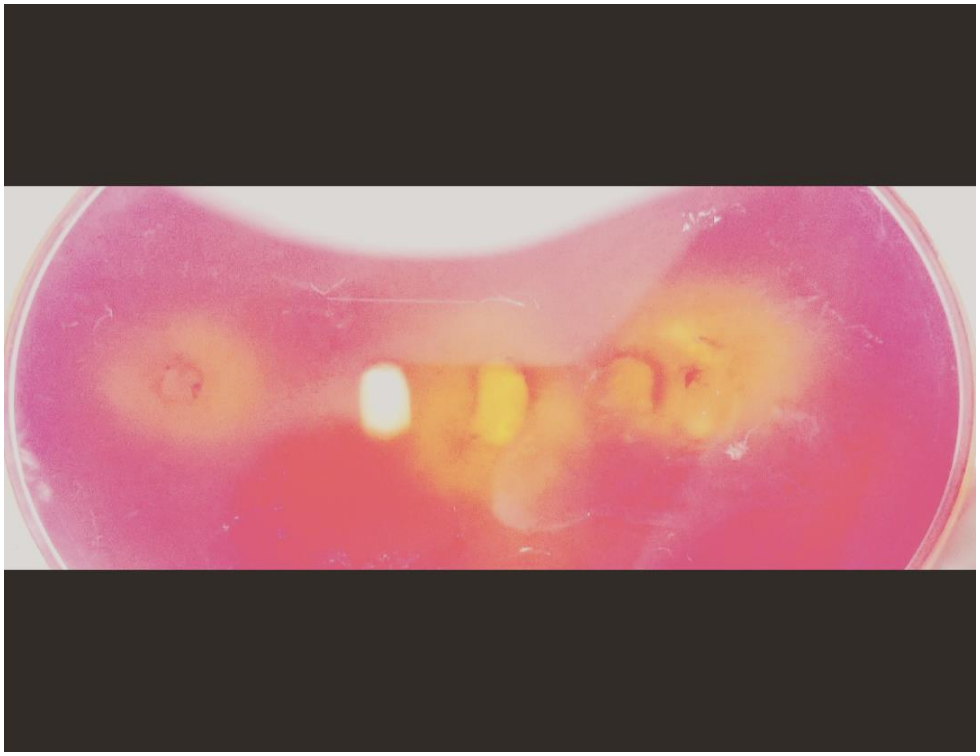

Plate 1: Cellulolytic ability as depicted by zone of clearance around *Pseudomonas* species on congo red supplemented medium

Discussion

A total of 10 bacteria and 7 fungal species were isolated from cassava processing mill soil and waste water from the steeped cassava. Organisms isolated were mostly fungi and few bacterial species (Tables 1 and 2). Fungi are mostly involve in biodegradation of cellulosic and hemicellulosic materials and are considered to be more efficient than bacteria in cases of degradation of these organic polymers (Ezeagu *et al*., 2023). Filamentous fungi utilize their hyphae for growth and nutrient absorption when in direct contact with the substrate upon which they are growing. This saprotrophic mode of fungal growth, ensures that the interwoven network of mycelia enter into the matrices of their substrate which is broken down through secretion of extra cellular enzymes. These unique features identifies fungal species as the major decomposers in forests soil and generally all ecological systems and environment (Maravi and Kumar, 2020; Suwannaphan *et al*., 2024*)*.

Complex organic matters are broken down into simple elements through enzymatic action for the fungal organism's growth and metabolic activities (Oboh, 2005; Ezeagu *et al*., 2023).The Fungal isolates belongs to the genera *Aspergillus (A. niger, flavus, terreus, fumigatus,* and an unidentified species), *Trichoderma*, and *Rhizopus* (Figure 1). The predominance of *Aspergillus* species in this study is in conformity with the work of Abu *et al*. (2000); Nwogwugwu *et al*., 2018; Ezeagu *et al*., 2023). *Aspergillus* species especially terreus exhibited high cellulolytic ability in this study, All species of *Aspergillus* have been reported for their abundance in the soil and enormous ability to produce different kinds of enzyme for biodecomposition in the soil, their ability to produce enzyme distinguishes them as novel organism for industrial enzyme production. The *Aspergillus* species are mostly considered for enzyme and organic acid production (Maravi and Kumar, 2020). Bhat (2000); Thygesen *et al.* (2003) documented similar findings during their research. This observation is also similar to previous studies carried out by Arotupin (2007), who stated that *Aspergillus niger* is a soil saprobe ubiquitously found in the environment, and capable of secreting wide array of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocelluloses. Cellulolytic ability of *Trichoderma* and *Rhizopus* has also been reported by Arotupin (2007) as a result of the possession of a genetic composition of a hydrolytic enzyme complex. The *Rhizopus* sp isolated demonstrated medium cellulase activity, this is in line with the work of Maravi and Kumar (2020). The bacteria isolates also demonstrated cellulase production ability at various levels, *Pseudomonas* was found to be the best while *Bacillus* was following closely. *Pseudomonas.* sp (Plate 1) and *Bacillus* sp. were observed to have high ability for cellulase production and they compete favourably with *A. terreus* and *niger* in this research. Arotupin (2007) confirmed that, *Pseudomonas* and *Bacillus* species isolated from steeped cassava exhibited a high capability to produce cellulase in large amounts. This might be attributed to the fact that *Pseudomonas* is a known bacteria with a complex enzyme system and ability to grow in many environment as well as produce many types of enzymes to enhance its survival in such ecosysyem (Maravi and Kumar, 2020; Suwannaphan *et al*., 2024). *Bacillus* species are another group of versatile bacteria of soil origin with high ability for enzyme production. Different species of this genus have been engaged and are still engaged in enzyme production especially cellulase. Previous authors isolated similar organisms to those reported in this study especially the bacteria species and most of the fungal isolates Nwogwugwu *et al*., 2018). The bacterial species were mostly able to ferment the hydrolysable sugar in the waste and soil of the cassava mill through the production of the celluase for growth and metabolism (Uzochukwu *et al*., 2001). In addition, species of *Bacillus*, *Aspergillus*, *Trichoderma*, and *Pseudomonas* have been reported to stand out as producers of cellulases (Haki and Rakshit, 2003; Oboh, 2005; Nwogwugwu *et al*., 2018).The poor cellulolytic activity observed with *Escherichia coli* might be due to the fact that the organism might not be authoctonous in the soil mill and waste environment but a contaminant of human origin. Cassava waste was reported to contain hydrolysable sugar, minerals, acid and or cyanogenic glucosides which might inhibit the growth of *E. coli* (Oboh and Akindahunsi, 2003). The bacteria and fungi isolated during this research demonstrated higher cellulolytic ability as shown by the diameter of zones of clearance than those reported by Nwogwugwu *et al*. (2018), this suggests that the isolates are more likely to be able to produce the cellulase for the local industries if they are considered for such. The differences obtained might be due to factors such as environmental variability, potential strain-specific challenges, or limitations in the experimental design. These factors can however be optimized in further research to enhance cellulase production.

CONCLUSION

The findings in the present study showed that cassava processing mill soil, and steeped cassava can be a very good source of industrially important organisms because of the ability as demonstrated in this research. The findings obtained in the present study as presented in Figure 1 strongly suggests the suitability of these species as good candidate for cellulase production. These bacterial and fungal species can be further exploited in the future for enzyme production. By harnessing the cellulolytic activity of native microbial population we could produce enough quantity of cellulase which will become a boom for our industries that are utilizing these enzymes for a number of processes (Ezeagu *et al*., 2018). However, limitation could exist under different experimental condition and genetic composition of strains.

RECOMMENDATIONS

From the findings in this research, it could be recommended that isolated strains of *Aspergillus terreus* and *Pseudomonas* be further explored for use in industrial enzyme production in microbiology base research firms. Furthermore, cassava waste water could be considered as potential source of important enzymes and hence could be converted into economic value through the development of technology for the safe collection of the waste water. Optimizing production processes, exploring genetic engineering, or conducting pilot studies for industrial application can further enhance the usability of these organisms.

REFERENCES

Abu E. A., Onyenekwe, P. C. Ameh, D. A. Agbaji,A. S. and Ado, S. A. (2000) "Cellulase production from sorghum bran by Aspergillus niger SL:1: an assessment of pretreatment methods," in *Proceedings of the International Conference on Biotechnology: Commercialization and Food Security (ICBCFS'00)*, pp. 153–159, Abuja, Nigeria.

Abu, E.A., Ado S.A., James, D.B. (2005). Raw starch degrading amylase production of mixed culture of *Aspergillus niger and Saccharomyces cerevisae* grown on Sorghum pomace, *Afr. J.Biotechnol*. 4; 785-790.

Akinrele, I.A., Cook, A.S. and Holgate, R.A. (2009).The manufacture of gari from cassava in Nigeria. In: *Proceedings, First International Congress on Food Technology*, London, PP. 633-644.

Aneja, K.R (1996) Experiments in microbiology, plant pathology, tissue culture and mushroom cultivation. New Delhi: New Age International Pvt. Ltd.

Anish, R., Rahman, M.S., and Rao, M. (2006). Application of cellulases from an alkalothermophilic *Thermomonospora* species in biopolishing of denims. *Biotechnology and Bioengineering* 96:48-56.

Arotupin, D. J. (2007). Evaluation of microorganisms from cassava waste water for production of amylase and cellulase. *Research J. Microbiol. 2*(5): 475 – 480.

Asgher, M., Asad, M.J., Rehman, S.U. and Legge, R.L., (2007). A thermostable α-amylase from a moderately thermophilic *Bacillus subtilis* strain for starch processing.*Journal of Food Engineering*, 79, 950-955.

Bahatkar, B. P., Gahukar, S. J., A. A. Akhare, A. A., Y. V. Ingle, Y. V., Rathod, D. R. and Charpe, A. M. (2023). Decomposition of Agriculture Farm Wastes by Cellulolytic Bacteria; International Journal of Environment and Climate Change; 13(10): 411-421, Article no.IJECC.104942; ISSN: 2581-8627

Balla, A., Silini, A., Cherif-Silini, H., Bouket, A.C., Boudechicha, A., Luptakova, L., Alenezi, F.N. and Belbahri, L. (2022). Screening of Cellulolytic Bacteria from Various Ecosystems and Their Cellulases Production under Multi-Stress Conditions; Catalysts, 12, 769. [CrossRef]

Bautista-Cruz, A., Aquino-Bolanos, T., Hernandez-Canseco, J. and Quinones-Aguilar, E.E. (2024). Cellulolytic Aerobic Bacteria Isolated from Agricultural and Forest Soils: An Overview. Biology, 13, 102. https://doi.org/10.3390/biology13020102

Bhat, M. K., (2000) "Cellulases and related enzymes in biotechnology," *Biotechnology Advances*, vol. 18, no. 5, pp. 355–383.

Buzzini, P. and Martini, A. (2002). Extracellular enzymatic activity profiles in yeast and yeast like strains isolated from tropical environments. J. Applied Microbiol. 93: 1020-1025.

Cappuccino, J.G. and Sherman, N. (2002). Microbiology : A laboratory manual. Pearson Education. 6 (4), 21-23, 138.
De Hoog, G.S., Guarro, J., Gene, J. and Figueras, M.J.(2000). *Atlas of Clinical Fungi.* Second edition. Central bureau voor Schimmel cultures. Utrecht.

Djoulde, R. D. (2004) Mise au point d'un ferment miste destine a la bioconversion des tubercules de manioc cyanogene. PhD thesis, L'Ecole Nationale Superieure des Sciences Agroindustrielles (ENSAI), Universite de Ngaoundere, Cameroun, 200pp.

Ezeagu, G.G., Sanusi, U.R., Wali, U.M. and Mohammed, S.S.D. (2023). Determination of Cellulolytic Potentials of *Aspergillus* Species Isolated from Central Waste Dump Site of Nile University of Nigeria, Science *World Journal*; 18(No 4) 2023 DOI: https://dx.doi.org/10.4314/swj.v18i4.13, www.scienceworldjournal.org, ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print

Fawole, M.O. and B.A. Oso, (2004). Characterization of Bacteria: *Laboratory Manual of Microbiology. 4th Edn., Spectrum Book Ltd., Ibadan, Nigeria, pp: 24-33.*

Gupta, P., Samant, K. and Sahu, A. 92012). Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. International Journal of Microbiology; DOI: 578925

Haki, G.D and Rakshith, S.K. (2003). Developments in industrially important thermostable enzymes; a review. *Bioresource Technology*. 89: 17-34.

Hayward, T.K., Hamilton, J. Tholudur, A. and McMillian, J.D. (2000).Improvements in titer productivity and yield using solka-floc for cellulase production. *Applied Biochemistry and Biotechnology*. 84-86: 859-874 http://www.nal.usda.gov/fnic/DRI//DRIEnergy/339-421.pdf.

International Institute for Tropical Agriculture, IITA (2005).Economic importance of cassava production in West Africa.*Integrated Cassava Project*, 10: 2321 – 2325.

Kobawila, S.C.,Louembe, D.,Keleke, S.,Hounhouigan, J. and Gamba, C. (2005).Reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntobambodi, two food products from Congo.*African Journal of Biotechnology*,4: 689-696.

Lynd, R.L., Weiner, P.J., Vanzyl, W.H and Pretorius, T.S. (2002) Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiology and Molecular BiologyReviews* 66: 506-577.

Malik,W.A. and Javed, S. (2021). Biochemical Characterization of Cellulase From *Bacillus Subtilis* Strain and Its Effect on Digestibility and Structural Modifications of Lignocellulose Rich Biomass. Front. Bioengineering and Biotechnology; 9, 800265.

Maravi, P. and Kumar, A. (2020). Isolation, Screening and Identification of Cellulolytic Bacteria from Soil, *Biotechnology Journal International*, 24(1): 1-8, 2020; BJI.54241, ISSN: 2456-7051.

Miyoko, H. and Henrik, B-F; (2000). Protein engineering of new industrial amylases. *Trends in Glycosccience and Glycotechnology* 12:389-401.

Oboh, G. (2005). Isolation and characterisation of cellulase from fermented cassava (Manihot esculenta Crantz) waste-water. *Afr. J. Biotech.4*(10):1117-1123.

Oboh G, Akindahunsi AA (2003). Biochemical changes in Cassava products (flour & gari) subjected to Saccharomyces cerevisaesolid media fermentation. Food Chem. 82(4): 599-602.

Oyeleke, S. B and Oduwole, A.A. (2009). Production of amylase by bacteria isolated from a cassava waste dumpsite

in Minna, Niger state, Nigeria. *African Journal of Microbiology Research* 3(4): 143-146.

Singh, A., Bajar, S., Devi, A. and Pant, D. (2021). An Overview on the Recent Developments in Fungal Cellulase Production and Their Industrial Applications; *Bioresources and Technological Representative*, 14, 100652.

Singh, S., Jaiswal, D.K., Sivakumar, N. and Verma, J.P. (2019). Developing Efficient Thermophilic Cellulose Degrading Consortium for Glucose Production From Different Agro-Residues, Frontier Energy Resources, 7, 61.

Thygesen, A., Anne, B.T. Schmidt, A.S., Henning, J, Birgitte,K.A. and Olsson, L. (2003). Production of cellulose and hemicellulose-degrading enzymes by filamentous fungi on wet-oxidised wheat straw. Enzyme and Microbial Technology, 32 :606-615.

Nwogwugwu, N.U., Abu, G.O., and Akaranta, O. (2018). Isolation of Cellulose Degrading Microorganisms from Agro-Wastes for Industrial Utilisation; *International Journal of Innovative Science, Engineering & Technology*, 5(2), ISSN (Online) 2348 – 7968; www.ijiset.com

Uzochukwu, S.A., Oyede, R.S. and Atanda, O. (2001). Utilization of cassava effluent in the preparation of gin, *Nigeria Journal of Microbiology*; 15: 87-92.