Isolation of Cellulolytic Fungi from Rice Husk

Christianah O. Jesubunmi a*, Precious O. Chinenyeye a, Charles C. Chigbu a and Joy O. Okechim a

a Department of Biological Sciences, Microbiology Programme, Clifford University, Ihie Campus, Owerrinta, Abia State, Nigeria.

ABSTRACT

Aim: To isolate cellulose degrading fungi from rice husk.

Study Design: The experiment was carried out under aseptic condition in 3 replicates.

Place and Duration of Study: Department of Biological Sciences, Microbiology Programme, Clifford University, Ihie Campus, Owerrinta, Abia State, Nigeria, between May 2021 to August 2021.

Methodology: Rice husk from a rice mill was collected and kept until visible microbial growth was noticed. The organisms were isolated, characterized and screened for enzyme activities.

Results: Cellulolytic organisms were isolated from rice husk, an agricultural by-product of rice milling. The isolates were identified as Penicillium and Aspergillus species. The two fungal isolates were screened for enzyme activity using 0.5 ml Carboxymethyl cellulose (CMC) as carbon source, the highest cellulase activity of 0.448 µg/ml/min was recorded for Aspergillus sp. at 48 hours while Penicillium sp, had enzyme activity of 0.388 µg/ml/min at day 1.

Conclusion: The result of this study shows that Aspergillus and Penicillium spp were isolated from rice husk and were able to secrete the enzyme cellulase which is a very important enzyme in so many industries and also very expensive. Thus isolating organism that can secrete this enzyme is an added advantage to many industries.

Keywords: Aspergillus; cellulase; enzyme production; Penicillium; rice husk.

*Corresponding author: E-mail: jesubunmic@clifforduni.edu.ng
1. INTRODUCTION

Rice husk, an agricultural by-product of rice milling, is abundantly available in Nigeria and has no direct nutritional value so it is left to rot or used as fuel. Rice husk when used in feeding birds resulted in poor growth performance as a result of its low nutritional quality, even though it has high fiber and lignin content [1].

Cellulose is the most abundant biological compound on terrestrial and aquatic ecosystem. It is the dominant waste material from agricultural industry in the form of stalks, stems and husk. Generally, cellulose is of great interest in its utilizing as an energy resource and feed, cellulose is composed of D-glucose units linked together to form linear chain via β-1, 4-glycosidic linkages [2]. This natural polymer has a linear structure, crystalline form and not easily to dissolve. Cellulose is one of the important additives to manufacture of bioplastics [3], food packaging materials [4], pharmaceutical, food, cosmetic and other industries [5].

Carbohydrate materials (sugars, starch and cellulose) are valuable and natural industrial raw materials used worldwide [6]. A lot of useful products can be produced from the monomeric units of these carbohydrate materials. However, in order to convert starch and cellulose to useful products, they need to be hydrolyzed into their monomeric units by either enzymes or chemicals (acids or bases). Although chemical hydrolysis is presently faster and cheaper than enzymatic method, it is not environmentally friendly and requires special (non-corroding) vessels for the reaction to take place. Bioconversion using enzymes are safer and more environmentally friendly than the use of chemicals [7].

Plant biomass contains cellulose as the major component of the cell walls. Cellulose accounts for 50% of the dry weight of plant biomass and approximately 50% of the dry weight of secondary sources of biomass such as agricultural wastes; cellulose is a strong fibrous, crystalline polysaccharide, resistant to hydrolysis and is water insoluble [8].

Cellulolytic enzymes play an important role in natural biodegradation processes in which plant lignocellulosic materials are efficiently degraded by cellulolytic fungi, bacteria, actinomycetes and protozoa. In industry, these enzymes have found novel applications in the production of fermentable sugars and ethanol, organic acids, detergents and other chemicals products. Cellulases provide a key opportunity for achieving tremendous benefits of biomass utilization. As important as this enzyme is, it is not readily available and also expensive therefore this study was designed to isolate organism that can secrete cellulase enzyme which will offer a major breakthrough for the industries where the enzyme has found usefulness.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The Rice Husk was collected from a Rice milling industry in Abakiliki, Ebonyi state, Nigeria.

2.2 Isolation of the Microorganisms from Rice Husk

10 g of rice husk was added to 90 ml of distilled water; 10 folds serial dilution was done with 0.1 ml each of 10-7, 10-8, 10-9 and 10-10 were plated out on Sabouraud dextrose Agar using pour plate method and was kept at room temperature for 7 days. Subculture of the growths were done on Sabouraud dextrose agar (SDA) and kept at room temperature for 7 days.

2.3 Identification of the Fungal Isolated Species

A slide culture (SDA) was prepared to identify the organism on the plate and after 7 days it was observed under the microscope. A small portion of the mycelia growth was carefully picked with a sterile inoculating needle and placed with a drop of lactophenol cotton blue on a microscope slide and covered with a cover slip. The slide was examined under the microscope, first with (x10) and then with (x40) objective lens for morphological examination. The isolates were further identified macroscopically using their cultural characteristics according to Gilma [9] and Barnett and Hunter [10].

2.4 Degradation Ability of Fungal Isolates on Carboxymethyl Cellulose Media (CMC)

Carboxymethyl cellulose (CMC) medium (See Appendix) was prepared and the fungal isolates were inoculated on the CMC media and incubated at room temperature for 7 days to check for the growth of the isolates.
2.5 Screening for Cellulase Activity

The isolates were grown in broth containing 1% CMC as carbon source. They were incubated at room temperature for 72 h, and then enzyme assay was carried out. The cellulase activity was measured.

2.6 Cellulase Assay

The method used involved estimating the amount of reducing sugar produced by the activity of the enzyme on buffered 1% CMC. The amount of reducing sugar produced was estimated using the dinitrosalicic acid (DNSA) method by Miller [11]. The reaction mixture containing 0.5 ml of supernatant and 0.5 ml of 1% CMC was incubated at 50 °C in a water bath for 30 mins. The reaction was terminated by adding 3 ml DNSA and then boiled for 10 min, in a boiling water bath. The control tubes contained the reaction mixture but lacked the crude enzyme solution. Absorbance was measured at 540 nm using a spectrophotometer [11]. The amount of reducing sugar produced was derived from a glucose concentration curve. The cellulase activity was measured by the release of reducing sugar over the period of biodegradation.

One unit of cellulase was defined as the amount of enzyme which released 1 µg of glucose from cellulose per ml per min under the assay conditions [12].

3. RESULTS AND DISCUSSION

3.1 Isolation and Screening of Cellulose Degrading Fungi

Few species of fungi were isolated on Sabouraud dextrose Agar medium and only two species were able to degrade cellulose. These fungal isolates were identified by cultural (See Appendix) and microscopic characteristics and were identified as Aspergillus sp. and Penicillum sp. The microscopic examinations are as shown in Figs. 1 and 2.

Fig. 1. Microscopic view of Aspergillus spp

Fig. 2. Microscopic view of Penicillum spp
Enzyme activities of *Aspergillus* and *Penicillium* are as shown in Figs. 3 and 4.

![Fig. 3. Enzyme activity of *Aspergillus* spp using 1% CMC as carbon source](image1)

![Fig. 4. Enzyme activity of *Penicillium* spp. using 1% CMC as carbon source](image2)

The two species of fungi (*Aspergillus* spp and *Penicillium* spp) were able to produce cellulase enzyme to degrade the cellulose content of rice husk. The result of this study is in agreement with the result of Oyeleke et al., [13], who isolated *Aspergillus niger* and other forms of bacteria from the gut of *Archahatina marginata* (Giant African Snail), also Edor et al. [1] conducted a study where *Aspergillus niger* synthesized cellulase, which biodegrade the cellulose content of rice husk.

In this study, *Aspergillus* spp has the highest enzyme activity at Day 2 (48 hrs), this agreed with the work of Oyeleke *et al.* who reported the highest enzyme activity for cellulase from *Aspergillus niger* at the same date. The highest cellulase activity of 0.448 µg/ml/min was recorded for *Aspergillus* sp. this is in agreement with Deka et al. [14] who reported 0.43 U cellulase activity of *Bacillus subtilis*. *Penicillium* sp, had cellulase activity of 0.388 µg/ml/min compared to *Penicillium* sp isolated by Jha et al. [15] with cellulase activity of 0.4427 U.

The present study demonstrated the presence of cellulolytic fungi with the capability to degrade rice husk and as a result prevent the accumulation of rice husk in the environment. Also the cellulase secreted by these fungi can be harnessed for many industrial purposes.
4. CONCLUSION

The result of this study showed that *Aspergillus* and *Penicillium* spp were isolated from rice husk and were able to secrete the cellulase enzyme. This chief enzyme in many industries is very expensive. Data found here could establish new perspectives in *Aspergillus* and *Penicillium* spp valorization in industrial purposes especially in agricultural, food, textile, pulp, paper and fermentation ones. However, the toxicity of these fungal species in such uses needs more investigations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Edor SP, Edogbanya OP, Kutshik JR. Cellulase activity of *Aspergillus niger* in the biodegradation of rice husk. MOJ Biology and Medicine. 2018: 3(2): 49-51.
2. Abedin IJ. Isolation and identification of cellulose degrading Bacteria from the soil sample. Department of Mathematics and Natural Sciences. 2015: 80.
3. Agustin MB, DeLeon ERP, Breunaobra JL, Alonzo SMM, Patriana MF, Hirose F, Ahmad B. Starch based bioplastics reinforced with cellulose nanocrystals from agricultural residues. International Conference on Advance in Engineering and Technology. 2014: 593-597.
4. De Moura MR, Mattoso LHC, Zucolotto V. Development of cellulose-based bactericidal nanocomposites containing silver nanoparticles as their use as active food packaging. Journal of Food Engineering, 2012: 109: 520-524.
5. Salehudia MH, Salleh E, Mamat SNH, Muhamed II. Starch based active packaging film reinforce with empty fruit bunch (EFB) Cellulose nanofibers. Procedia Chemistry. 2014: 9: 23-33.
6. Sudha PN, Aisverya S, Nithya R, Vijayalakshmi K. Industrial applications of Marine carbohydrates. Advance Food Nutrition Resource. 2014: 73: 143-181
7. Ogbonna CN, Nnaji OB, Chioke OJ. Isolation of amylase and cellulase producing fungi from decaying tubers and optimization of their enzyme production in Solid and Submerged cultures. International Journal of biotechnology and Food Science. 2018: 6(1): 9-17.
8. Rawway M, Salah GA, Ahmed SB. Isolation and Identification of cellulose degrading bacteria from different sources at Assiut Governorate (Upper Egypt). An International Journal of Ecology of Health and Environment. 2018: 6(1): 15-24.
9. Gilman CJ. A manual of soil fungi. Lower State College Press: Univ Park. 1982: 216.
10. Barnett HL, Hunter BB. Illustrated Genera of Imperfect Fungi. Burgess. Pub. Co., Minnesota, USA.1972: 241.
11. Miller GL. Use of Dinitrosalycylic acid reagent for determination of reducing sugar. Analytical Chemistry. 1959: 31: 426-428.
12. Ojima T, Inoue A. methods in Enzymology. Science Direct. 2018: 605: 457-497.
13. Oyeleke SB, Egwim EC, Oyewole OA, John EE. Production of Cellulase and protease from Microorganisms Isolated from Gut of *Archachatina marginata* (Giant African Snail). Science and Technology. 2012: 2(1): 15-20.
14. Deka D, Bhargavi P, Sharma A, Goyal D, Jawed M, Goyal A. Enhancement of cellulose activity from a new strain of Bacillus subtilis by medium optimization and analysis with various celluloscopic substrates. Enzyme Research. 2011: Article ID 151656: 1–8.
15. Jha VK, Prasad B, Pranay K. Screening and isolation of cellulose producing fungal strains. Korean Journal of Microbiology and Biotechnology. 2016: 6(6): 2231–3168.
APPENDIX

Carboxymethyl cellulose (CMC) Medium Composition:

| Ingredient  | Quantity  |
|-------------|-----------|
| FeSO₄.7H₂O  | 0.004 g/l |
| NaNO₃      | 3 g/l     |
| NaHPO₄     | 1 g/l     |
| MgSO₄      | 0.1 g/l   |
| KCl         | 0.5 g/l   |
| CMC         | 10 g/l    |

Plate 1. *Aspergillus* spp on sabourad dextrose agar media

Plate 2. *Penicillium* spp on sabourad dextrose agar media
Plate 3. *Aspergillus* spp on carboxymethyl cellulose media

Plate 4. *Penicillium* spp on carboxymethyl cellulose media

© 2022 Jesubunmi et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/83320