Isolation of Cellulolytic Fungi from Rice Husk

ABSTRACT

Aim: Isolation of cellulose degrading fungi from rice husk

Study Design: The experiment was carried 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* sp. The two fungal isolates were screened for enzyme activity using 0.5ml Carboxymethyl cellulose (CMC) as carbon source, the highest enzyme activity of 0.448µg/ml/min was recorded for *Aspergillus* sp. at 48 hours while *Penicillium* sp, had enzyme activity of 0.388µg/ml/min at day 1.

Keywords: *Aspergillus niger,* Cellulase, Enzyme production, *Penicillium*, Rice husk.

1. INTRODUCTION

Rice husk, an agricultural by-product of rice milling, is abundantly available in Nigeria and has no direct nutritional value and used in ~~so it is left to~~ rot or ~~used~~ as fuel. An attempt at feeding it to poultry resulted in poor growth performance as a result of low nutritional quality, though high in 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 ~~there has been~~ great interest in its utilizing ~~cellulose~~ as an energy resource and feed, the cellulose is composed of D-glucose units linked together to form linear chain via ß-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, foods, cosmetic and other industries [5]. ~~Cellulose is a natural polymer having a linear structure, crystalline form and not easily to dissolve.~~

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].

Comment [Q1]: Add reference

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 expensive, therefore this study was designed to isolate organism that can secrete cellulase~~the enzyme as~~ wich ~~this~~ will ~~be a~~ 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 ~~weighed~~ added to~~into~~ 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 ~~Isolates~~isolated species

A slide culture was prepared to identify ~~the~~ isolated organisms on the plate and after 7 days it was observed under the microscope. A small portion of the mycelia growth was carefully picked with ~~the aid of~~ a sterile inoculating needle and placed ~~in~~ 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) objectives lens for morphological examination. The isolates were further identified macroscopically using their cultural characteristics according to Gilma [9] and Barnett and Hunter [10]

Comment [Q2]: Add Culture medium name and composition

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

Carboxymethyl cellulose (CMC) medium was prepared and the fungal isolates were inoculated on the CMC media for 7 days to check for the growth of the isolates.

Comment [Q3]: Add composition

Comment [Q4]: Add temperature of incubation

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 than ~~, after which enzyme~~an 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 ^{0}C in a water bath ~~for~~ during 30 min~~s~~. The reaction was terminated by adding 3 ml DNSA and then boiled for 10 min~~s~~ in a boiling water bath. The control tubes contained the reaction mixture but lacked the crude enzyme solution. Absorbance was ~~taken~~ measured at 540 nm using a spectrophotometer ()[11]. The amount of reducing sugar produced was derived from a glucose concentration curve.

Comment [Q5]: Add type of material

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.

Comment [Q6]: Add reference

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 (Appendix) and microscopic characteristics and were identified as *Aspergillus* sp. and *Penicillum* sp. The microscopic examinations are as shown in Figures 1 and 2.

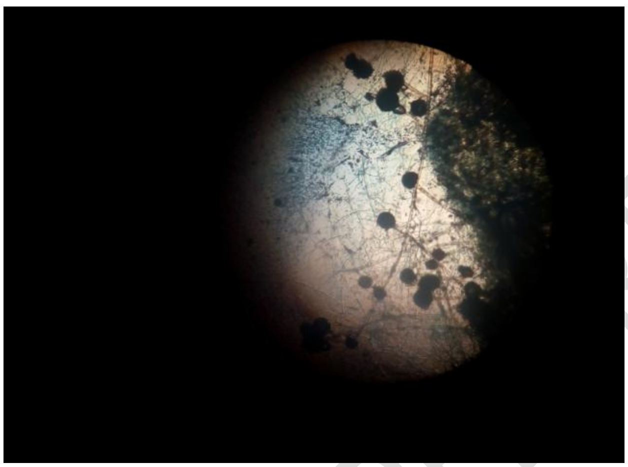

Figure 1: Microscopic View of *Aspergillus Sp.*

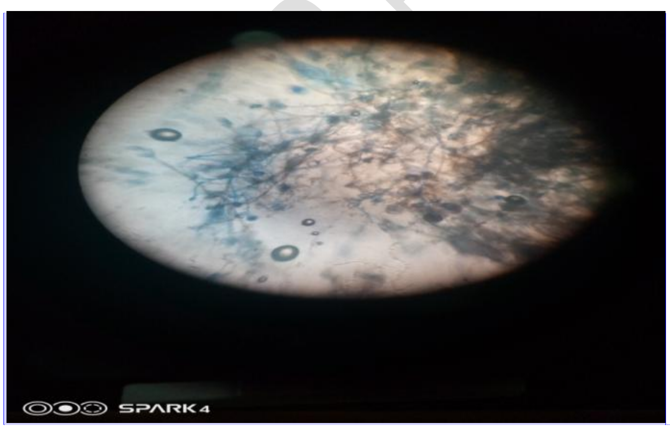

Figure 2: Microscopic view of *Penicillium* sp

Comment [Q7]: This two figures need to be changed with other with best quality, resolution. They are not clear we can't observe spore and hyphae
Add scale used in figures

Enzyme activities of *Aspergillus* and *Penicillium* are as shown in Figures 3 and 4.

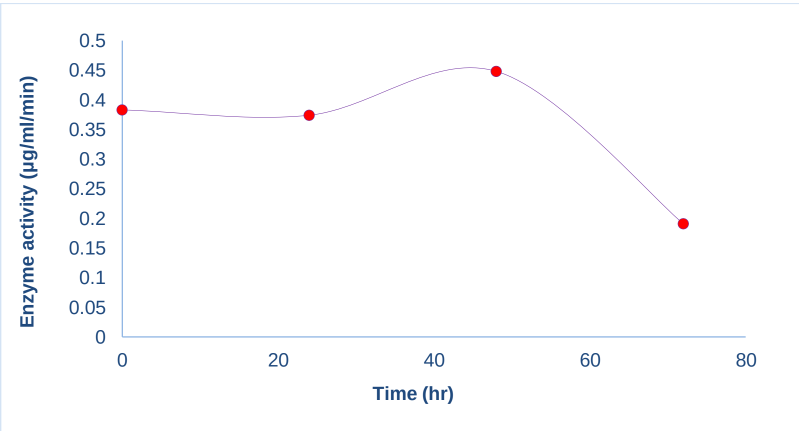

Figure 3: Enzyme Activity of *Aspergillus sp.* using 1 % CMC as carbon source

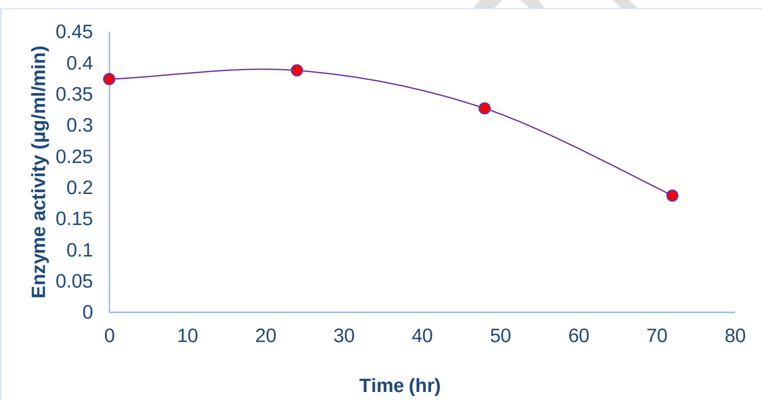

Figure 4: Enzyme Activity of *Penicillium ~~Sp~~sp.* using 1 % CMC as carbon source

The two species of fungi (*Aspergillus* sp. and *Penicillum* sp.) were able to produce cellulase enzyme to degrade the cellulose content of rice husk. The result of this study ~~is in~~is in agreement with the result of Oyeleke *et al.,* [12], who isolated *Aspergillius niger* and other forms of bacteria from the gut of *Archachatina ~~Marginata~~ marginata* (Giant African Snail).~~,~~ ~~also~~Also Edor *et al*., [1], conducted a study where *Aspergillus niger* synthesized cellulase which biodegrade the cellulose content of rice husk. The cellulase activity was measured by the release of reducing sugar over the period of biodegradation.

In this study, *Aspergillus* sp. has the highest enzyme activity at ~~Day~~ day 2 (48 hrs), this agreed with work of Oyeleke *et al*.,[12] who reported the highest enzyme activity for cellulase from *Aspergillus niger* at the same date~~Day 2~~.

The result of this study has demonstrated the isolation of cellulolytic fungi from rice husk, these organisms ~~can~~ cauld be useful in ~~so~~ many industries ~~there~~by reducing the cost of production and ~~also reducing~~ the organic waste in the environment.

Comment [Q8]: Add cellulose amount produced with this two fungal species
And discuss your values with other organisms used in industries (with example in food, agriculture fuel…)

Comment [Q9]: Add discussion with *Aspergillus* and *Penicillium* sp toxicitu in foods industry and the quantity to use to guarantee enzyme production without a toxicity risk

4. CONCLUSION

The result of this study ~~shows~~ showed that *Aspergillus* and *Penicillium* sp. ~~can secrete~~produce the ~~enzyme~~ cellulase enzyme. This chief enzyme ~~which is a very important enzyme~~ in ~~so~~ many industries is ~~and~~ very expensive. Data founded here could establish neew perspectives in *Aspergillus* and *Penicillium* sp valorization in ~~Thus isolating organism that can secrete this enzyme is an added advantage to industries~~ industrial purposes especiallyin ~~like the~~ Agricultural ~~industries~~, food ~~industries~~, textile ~~industries~~, pulp, ~~and~~ paper ~~industries,~~ and fermentation ~~industries.~~ones. However, toxcisity of these fungal species in such uses nedd more investigations

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Comment [Q10]: pages

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APPENDIX

Comment [Q11]: put this figure in results part

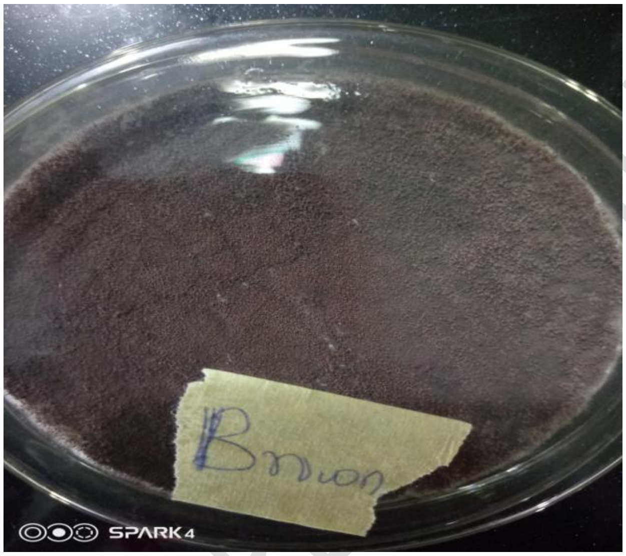

Plate 1 *Aspergillus sp.* On Sabourad Dextrose Agar media

Plate 2 *Penicillium sp.* On Sabouraud Dextrose Agar media

Plate 3 *Aspergillus sp.* On Carboxymethly Cellulose Media

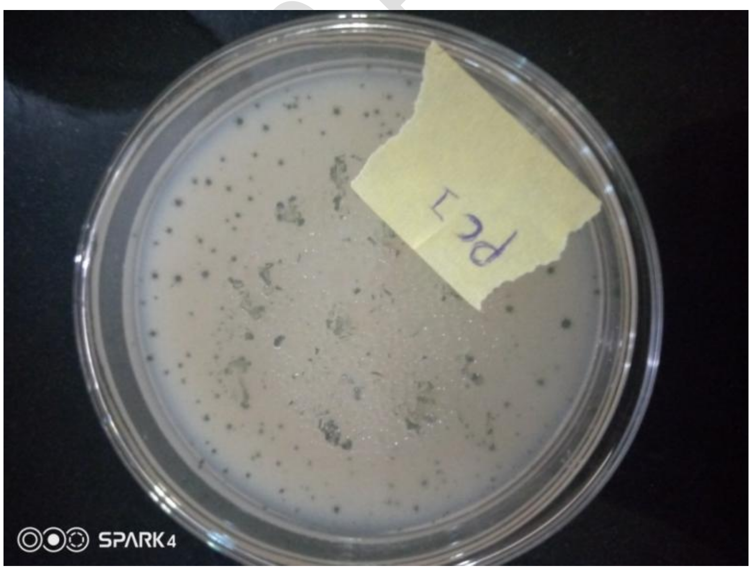

Plate 4 *Penicillium sp.* On Carboxymethyl Cellulose media