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ALPHA – AMYLASE AND GLUCOAMYLASE ACTIVITY IN SUBMERGED FERMENTATION OF RICE MILL FEED

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ABSTRACT

The *α*-amylase and glucoamylase activity of three fungi (*Aspergillus niger, Trichoderma viride* and *Rhizopus oryzae*) in mono and mixed culture was determined under submerged fermentation (SmF) process. Rice mill feed (RMF); an agro-industrial by-product of rice milling was used as substrate.

Fifty (50) ml of a basal medium containing 0.5gm of RMF in 150ml Erlenmeyer flasks were prepared in triplicates for each fungus and their mixed culture. Sterilization was carried out by autoclaving at 121ºC for 15 mins and 15psi. The flask was allowed to cool before inoculating with 3ml of inoculums and incubated for 7 days at 30ºC±2ºC. The flasks were placed on a rotary shaker (80rpm) for one hour daily during incubation. At the end of the incubation period, the contents of each flask were filtered through Whatman number 1 filter paper and taken as crude enzyme extract for *α* amylase and glucoamylase activity determination.

The *α* amylase activity recorded in single culture SmF of RMF was significantly higher ($p<0.05$) in *Aspergillus niger* (20.67 Iu/ml) compared to *T. viride* (13.00 Iu/ml) and *R. oryzae* (12.00 Iu/ml). Glucoamylase activity recorded wit *A. niger* under SmF was numerically (20.33Iu/ml) higher than values recorded with *T. viride* (15.57Iu/ml) and *R. oryzae* (18.57Iu/ml). Glucoamylase and *α*-amylase activity was significantly ($p<0.05$) higher in mixed culture of all 3-fungus compared to the other mixed culture treatments. Significantly higher ($p<0.05$) glucoamylase activity (48.00 Iu/ml) was expressed when all three fungi were present followed by the mixed culture of *A. niger* + *R. oryzae* (33.00Iu/ml).

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In conclusion, mixed fungi culture SmF of rice mill feed yielded better *α*-amylase and glucoamylase activity than single culture SmF process. Rice mill feed has potential as a suitable substrate for *α*-amylase and glucoamylase production.

Keywords: agro-industrial by-product, α-amylase, fungi, glucoamylase, rice mill feed, submerged fermentation

1. INTRODUCTION

The enzymes α-amylases, β-amylases and glucoamylases are starch hydrolyzing enzymes put to use in the starch processing industries for the hydrolysis of starch into simple sugar fractions by degrading 1-4 linkage of starch. Also described as amylases, these enzymes are utilized in numerous commercial processes such as thinning and liquefaction of starch in the alcohol, brewing and sugar industries (Sanghvi *et al.*, 2011). They also have numerous applications in bread and baking industry, starch liquefaction and saccharification, paper and detergent industry. According to Metin *et al.* (2010), amylases are used for analysis in medical and clinical chemistry, in the food and pharmaceutical industries. Of recently, α-amylases, β-amylases and glucoamylases denote one of the most important enzyme groups within the field of biotechnology (Bansode, 2010). Starch hydrolytic enzymes comprise 30 % of the world's enzyme consumption (Sanghvi *et al.*, 2011). Current findings on the use of microorganism as sources of industrially relevant enzymes have led to an enlarged awareness in the application of microbial enzymes in numerous industrial processes (Varalakshmi *et al.*, 2009). Today a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry (Gupta *et al.*, 2003). A read-through of literature indicates that amylases of fungal derivation are more stable than those of bacterial origin (Sanghvi *et al.*, 2011).

Several agro-industrial by-products are utilized for the synthesis of enzymes either through solid state fermentation or submerged fermentation procedure. Although solid state fermentation (SSF) is opined to yield better results, however, this does not rule out the benefits and usefulness of enzyme quantity and bioactive compounds produced using submerged fermentation (SmF) procedures (Subramaniyam. and Vimala, 2012). The one-step milling of rice results in rice mill feed (RMF), multiple step milling of rice yields rice bran which can result in the generation of 5 – 8% rice bran (Oliveira *et al*., 2010). Rice mill feed (RMF) is a cheap agro-industrial by-product from the one-step milling of rice (*Oryza glaberrima*).

Compared to rice bran from multiple processing of rice, RMF contains less starch, carbohydrates, protein, lipids and crude fibre (Ofongo *et al.*, 2008; Oliveira *et al*., 2010; Kupski *et al*., 2012; Chutmanop *et al.*, 2012). According to Chutmanop *et al.* (2012) rice bran provides an excellent medium for enzyme production through solid state fermentation with a

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carbohydrate/protein ratio of 2-3. Based on the concentration of crude fibre in RMF, and low carbohydrate concentration, its potential as a substrate for α-amylase and glucoamylase synthesis may be limited.

The aim of this study, was to determine the α-amylase and glucoamylase activities of selected fungi (*Aspergillus niger*, *Trichoderma viride* and *Rhizopus oryzae*) during the submerged fermentation of rice mill feed.

2. MATERIALS AND METHODS

The RMF used in this study was purchased from a rice milling plant in Ilorin Kwara state,

Nigeria. The mill was a one-step mill, meaning that the husk contained the bran, ground hulls and some broken rice.

2.1 Experimental site

The Submerged Fermentation experiment for enzyme activity determination was carried out at the Botany and Microbiology Department of the University of Ibadan.

2.2 Source of fungi

Pure cultures of *Aspergillus niger*, *Trichoderma viride* ad *Rhizopus oryzae* were obtained from the culture bank of the Microbial Physiology Laboratory, Department of Botany and Microbiology, University of Ibadan. They were sub-cultured on Potato Dextrose Agar (PDA) to obtain fresh inoculum for this study.

2.3 Determination of Inoculum size

The inoculum size was determined according to the method of Ofongo *et al.* (2018)

2.4 Determination of enzymes synthesized

A modified version of Mandel and Weber (1969) medium as developed by Hatakka and Pirhonen (1985) was used as the basal medium. 50 ml of the basal medium and 0.5gm of RMF in 150ml Erlenmeyer flasks were prepared in triplicates for each fungus and their mixed culture. Sterilization was carried out by autoclaving at 121ºC for 15 mins and 15psi. The flask was allowed to cool before inoculating with 3ml of inoculums and incubated for 7 days at 30ºC±2ºC.

The basal medium used contained per litre KH_2PO_4 (2.0gm); $(NH_4)_2SO_4$ (2.1gm); $MgSO_4.7H_2O$ (0.3gm); $CaCl_2.6H_2O$ (0.3gm); $MgSO_4.H_2O$ (1.56mg); $ZnSO_4.7H_2O$ (1.4mg); $CoCl_2.6H_2O$ (2.66mg); yeast extract (0.5gm) all regulated to a pH of 5.0.

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The inoculum used was prepared with a 1× 6 cork borer of each fungus in (mono and mixed culture) from 7 days old culture plates dispersed in sterile deionized water. During the period of incubation, the flasks were placed on a rotary shaker (80rpm) for one hour daily. At the end of the incubation period, the contents of each flask were filtered through Whatman number 1 filter paper. The filtrate was used to determine the following enzymes:

1. α-amylase: according to the method of Mandels (1974).
2. Glucoamylase: according to the method of Mandels (1974).

2.5 Experimental design and statistical analysis

Completely randomized design was used for the experiment having seven treatments and three replicates per treatment. Data obtained were subjected to one-way Analysis of Variance (ANOVA) and significant means separated by Duncan Multiple Range Test using SPSS version 17 (SPSS Inc, Chicago, USA).

3. RESULTS AND DISCUSSION

The enzyme activities (Iu/ml) of *Aspergillus niger*, *Trichoderma viride* and *Rhizopus oryzae* during submerged fermentation of rice husk is presented in figure 1 (pure culture of each individual fungus) and figure 2 (mixed culture SmF of rice mill feed).

alpha amylase and glucoamylase activity in single culture fungi fermentation of rice mill feed (RMF)

Amylase can be obtained from several fungi, yeast, bacteria and actinomycetes; however, especially fungi, have gained much attention because of the availability and high productivity of fungi, which are also amenable to genetic manipulation (Sidkey *et al*., 2011).

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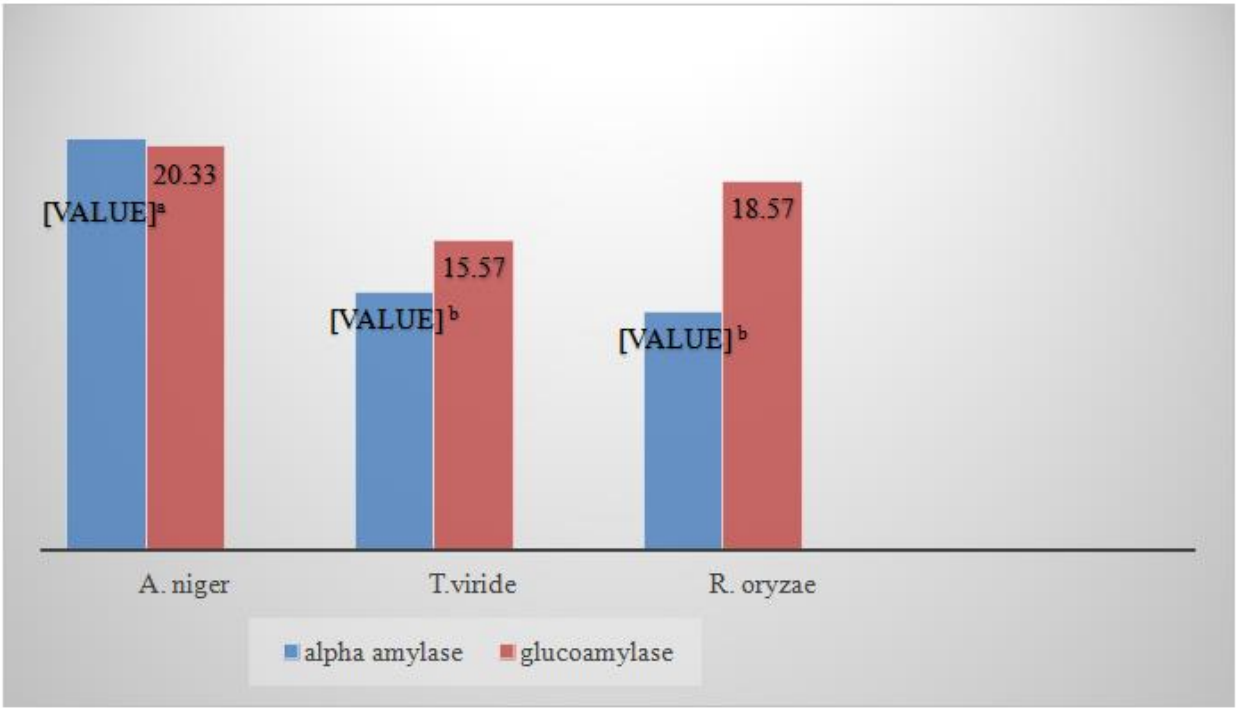

Figure 1: Enzyme activity in pure fungus culture fermentation (Iu/ml) (n=3)

Many fungi had been found to be good sources of amylolytic enzymes. The α amylase activity recorded in single culture SmF of RMF was significantly higher ($p \leq 0.05$) in *Aspergillus niger* (20.67 Iu/ml) compared to *T. viride* (13.00 Iu/ml) and *R. oryzae* (12.00 Iu/ml), which is not significantly different ($p \geq 0.05$) among each other. Ferreira *et al*. (2015) reported a high enzyme activity for a thermotolerant strain of *R. oryzae* using wheat bran as substrate. The value of glucoamylase recorded in this study was not significantly ($p \geq 0.05$) different across the 3 fungi treatment. Glucoamylase activity recorded wit *A. niger* under SmF was numerically (20.33Iu/ml) higher than values recorded with *T. viride* (15.57Iu/ml) and *R. oryzae* (18.57Iu/ml). Earlier work with *A. niger* indicated its better amylase activity compared to other fungi. This was reported by Abalaka, and Adetunji (2017), that *Aspergillus niger* had the largest zone of amylase activity (35.0 mm) when compared to *Fusarium pallidorosium* that had the lowest (5.0 mm) zone of amylase activity. The study of Ferreira *et al*. (2015) showcased the potential of a thermostable *R. oryzae* in synthesizing α-amylase under SSf conditions. The results of these studies (Ferreira *et al*. 2015; Abalaka, and Adetunji, 2017) may be suggestive of the numerically higher α-amylase activity recorded with *A. niger* and *R. oryzae* in this study.

Enzyme activity in mixed culture fungi fermentation of RMF

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Alpha amylase and glucoamylase activities of mixed culture of *Aspergillus niger*, *Trichoderma viride* and *Rhizopus oryzae* during submerged fermentation of RMF is presented in figure 2. Glucoamylase and α-amylase activity was significantly ($p<0.05$) higher in mixed culture of all 3-fungus compared to the other mixed culture treatments. Values recorded were; 39.67 Iu/ml (α-amylase) and 48.00 Iu/ml (glucoamylase) respectively. Glucoamylase activity was highest in *A. niger*, but these apparent differences were not significantly different ($p>0.05$) in single fungi submerged fermentation of RMF.

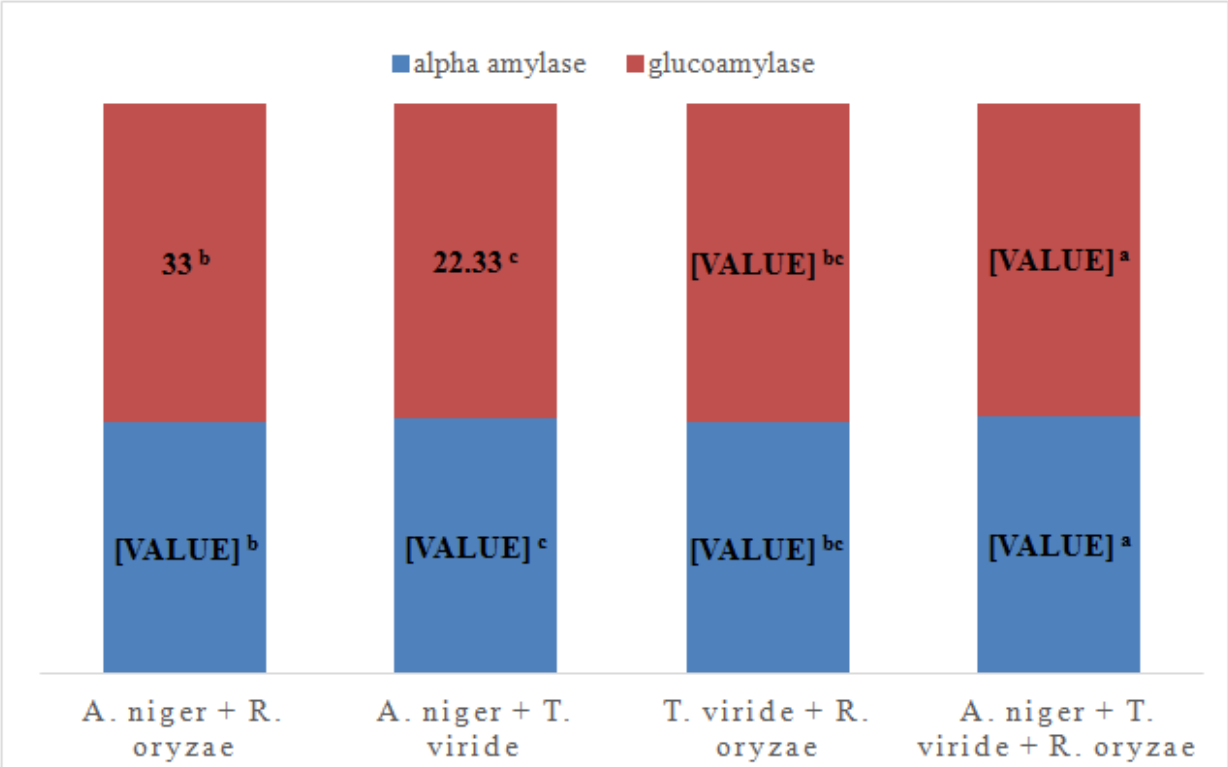

Fig. 2: alpha amylase and glucoamylase activity (iu/ml) in mixed fungi culture (n=3)

However, in mixed culture, significantly higher ($p<0.05$) glucoamylase activity (48.00 Iu/ml) was expressed when all three fungi were present followed by the mixed culture of *A. niger* + *R. oryzae* (33.00Iu/ml). The amylase activity recorded in the mixed culture of *A. niger* and *R. oryzae* is not out of pace since both fungi have been reported to yield high activity of amylases from literature (Ogbonna *et al*. 2014; Ferreira *et al.* 2015; Abalaka and Adetunji, 2017). According to Zambare (2010); glucoamylase is produced exclusively by the genus *Aspergillus.* the author further stated that, optimized glucoamylase activity was recorded with wheat bran

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having the highest enzyme production (1602U/gram of dry fermented substrate - gdfs) followed by rice bran with a value of 1271 U/gdfs. Rice husk was reported to yield almost same units of glucoamylase (875U/gdfs) with cotton seed powder (Zambare, 2010).

Production of very high levels of a hard starch-gel digesting amylo-glucosidase under SSF using wheat bran, rice bran, other rice components and combination of these was also reported by Singh and Soni (2001). These substrates are known for their starch content which could be a contributory factor to enzyme activities recorded when utilized as substrate for α-amylase and glucoamylase production. This further buttress the impact of substrate availability on products of fermentation either under solid state or submerged fermentation processes. In addition, inoculum size in mixed culture fermentation is another likely factor responsible for values recorded in this study. Earlier reports suggest that high inoculum level resulted in higher enzyme activity recorded (Zambare, 2010 and Kunamueni *et al.,* 2005). High concentrations of spores were attributed for increased enzyme production because of higher substrate specificities coupled with substrate availability. Furthermore, according to Banjo *et al.* (2014), amylase production by mixed culture of *Aspergillus flavus* and *Aspergillus tamarii* showed a pronounced synergy between the two moulds at a temperature of 70° Celsius at pH of 6.0 and 7.0 respectively. Under these conditions the enzyme activity of the mixed culture was reported to be 2.5times higher than that of the monocultures. Invariably, pH and temperature do play an additional role in impacting enzyme activity.

4. CONCLUSION

It is evident from the results of this study that the submerged fermentation of RMF demonstrated using three indigenous fungi (*Aspergillus niger, Trichoderma viride* and *Rhizopus oryzae*) both in pure and mixed culture yielded *α*-amylase and glucoamylase. Mixed culture submerged fermentation yielded better α-amylase and glucoamylase activity compared to single culture of each individual fungi.

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