

Evaluation of Ten Wild Nigerian Mushrooms for Amylase and Cellulase Activities

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Amylases and cellulases are important enzymes that can be utilized for various biological activities. Ten different wild Nigerian mushrooms (*Agaricus blazei*, *Agaricus* sp., *Coriolopsis occidentalis*, *Coriolus versicolor*, *Termitomyces clypeatus*, *Termitomyces globulus*, *Pleurotus tuber-regium*, *Podoscypha bolleana*, *Pogonomyces hydnooides*, and *Nothopanus hygrophanus*) were assayed for production of these secondary metabolites. The results revealed that most of the tested wild fungi demonstrated very good amylase and cellulase activities. With the incorporation of carboxymethyl-cellulose (a carbon source) into the culture medium, *Agaricus blazei* had the highest amylolytic activity of 0.60 unit/mL (at 25°C, pH 6.8). This was followed in order by *P. tuber-regium* and *Agaricus* sp. with 0.42 and 0.39 unit/mL, respectively ($p \leq 0.05$). Maltose and sucrose supplementation into the submerged liquid medium made *N. hygrophanus* and *P. hydnooides* to exhibit very low amylase activities of 0.09 and 0.11 unit/mL, respectively. Introducing peptone (an organic nitrogen source) into the basal medium enhanced the ability of *C. versicolor* to produce a cellulase value of 0.74 unit/mL. Other organic nitrogen sources that supported good cellulase activities were yeast extract and urea. Sodium nitrate (inorganic nitrogen source) generally inhibited cellulase production in all mushrooms. The best carbon source was carboxymethyl-cellulose, which promoted very high cellulase activity of 0.67 unit/mL in *C. versicolor*, which was followed in order by *P. tuber-regium*, *T. chypeatus*, and *C. occidentalis* ($p \leq 0.05$). Sucrose was the poorest carbon compound, supporting the lowest values of 0.01, 0.01, and 0.14 unit/mL in *P. hydnooides*, *A. blazei*, and *Agaricus* sp., respectively.

KEYWORDS : Enzyme activities, Nigeria, pH, Temperature, Wild mushrooms

In the developing countries of the world such as Nigeria, uncontrolled population growth has created problem of limited food supply, which has led to search for new methods to provide adequate food for humans. Over three-quarters of the population in under-developed countries consume < 30 g of protein/person/day, which is half of the recommended dose [1, 2]. This occurs because the cost of meat and fish are beyond the reach of the average citizen. Most people in underdeveloped and developing countries in Africa cannot afford these essential food commodities, as a result of low per capita income. This problem has led to malnutrition and various diseases, particularly in children. Therefore, methods to source alternative protein for common people are needed. Edible mushrooms are considered a valued delicacy that contribute to good nutrition such as protein, fibre, carbohydrates, vitamins and minerals to the human diet [3-6]. Mushrooms are highly nutritious and are an important food in many countries [7-10]. Mushrooms are saprophytes or parasites that are usually found among the Basidiomycetes and Ascomycetes classes of fungi. Generally, they can use various agro-industrial wastes as growing substrates [11-

13]. The majority of mushroom lovers in developing countries depend solely on mushrooms that grow in the wild. Artificial cultivation of mushrooms begins with spore germination, mycelial colonization, spawn production, and the development of fruiting bodies [14]. During these stages of mushroom development, mycelia usually secrete enzymes into the substrate, which breaks down the substrate into simple soluble organic compounds that can be transported into the hyphae where they are utilised for metabolic activities. During development, mycelia intertwine with themselves and colonise the substrates to develop fruiting bodies [15].

Enzymes occur in all living things, including mushrooms. Every living cell produces large number of enzymes for hydrolysis; oxidation, reduction, or metabolism [15, 16]. Enzymes usually play significant roles in mushroom development, and they also affect nutrient value, flavour, and shelf-life of these fungi [2].

Some important enzymes that have been reported in mushroom fruiting bodies and mycelia are cellulase, amylase, glutamate dehydrogenase, laccase, isocitrate lyase, and cytochrome. Different amylases and cellulases from

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various fungal sources have largely been screened and studied for their commercial utility [16, 17]. Mushroom mycelia, growing under submerged liquid media, can be used in enzyme enumeration studies [18]. Cellulolytic enzymes play important roles in natural biodegradation processes. These extra-cellular enzymes usually aid in converting lignocellulosic materials into soluble substances, which can be absorbed for vegetative growth and fruiting body development. Hydrolytic enzymes such endo- β -1,4 glucanase, exo- β -1,4 glucanase and β -1,4 glucosidase are involved in the conversion of cellulose to glucose [2]. Microbial enzymes have been employed in food, pharmaceutical, textile, paper, leather and other related industries and in biodegradation of cellulolytic materials [19]. The objective of this study was to optimise cultural conditions for amylase and cellulase production in wild collected mushrooms from southwestern Nigeria using submerged liquid media.

Materials and Methods

Test samples. The mushrooms used were collected from secondary forests in the Akoko area of Ondo, Nigeria. The collected samples were *Agaricus blazei*, *Agaricus* sp., *Corilopsis occidentalis*, *Coriolus versicolor*, *Termitomyces clypeatus*, *Termitomyces globulus*, *Pleurotus tuberregium*, *Podoscypha bolleana*, *Pogonomyces hydnooides*, and *Nothopanus hygrophanus*.

Enzyme assays. Sporophores of collected mushrooms were tissue cultured to generate mycelial starter cultures, which were established on potato dextrose agar supplemented with 5% yeast extract for 5 days at $28 \pm 2^\circ\text{C}$ [14]. They were then sub-cultured onto starch-yeast-extract broth medium [2]. The filtrates of each fungus were assayed for amylase using the modified dinitrosalicylic acid (DNSA) reagent method of Zhou *et al.* [20]. The amount of reducing sugar that was released was determined by adding 1 mL of DNSA to 1 mL of filtrate-starch-reaction mixture, and the absorbance was read at 540 nm using a spectrophotometer. Cellulase activity in the filtrate was determined by the method of Jahangeer *et al.* [21]. The assay medium contained 0.55% carboxymethyl cellulose (CMC) in 0.55 M acetate buffer (pH 6.8), and the reducing sugars released were measured by the DNSA reagent method of Parra *et al.* [22].

Effect of pH. Thirty mL of the chemically defined medium [14] was dispensed into 100 mL conical flasks, the pH was adjusted to 3.8, 4.8, 5.8, and 6.8 and the media was sterilised. The flasks were inoculated with actively growing mushroom mycelial cultures (5-day-old) using a sterile cork borer (6 mm diameter), and the cultures were incubated at $28 \pm 2^\circ\text{C}$ for 7 days. Enzyme

activity was determined by filtering the culture to remove the mycelia and measuring the activity of the mycelial free filtrate [20, 21].

Effect of temperature. The same chemically defined medium used for the pH measurements was used. The incubation was conducted at 25, 30, 35, and 40°C for 7 days, and enzyme activity was determined as described previously [20, 21].

Effect of carbon source. Each test fungi was cultured separately in a chemically defined medium (yeast extract, 2.5 g; KH_2PO_4 , 0.05 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 g; $\text{FeSO}_4\text{KNO}_3$, 1.55 g and 1,000 mL of deionised water). The carbon sources included CMC, glucose, maltose, and sucrose. Thirty mL of the carbon supplemented liquid medium was dispensed separately into 100 mL conical flasks and sterilised. After cooling, each flask was inoculated with mycelial culture of the specific test fungus using a sterile cork borer (6 mm diameter) and incubated at $28 \pm 2^\circ\text{C}$ for 7 days [14]. Enzyme activity was determined by filtering the culture to remove the mycelia and used as a mycelial free filtrate using DNSA reagent [20, 21].

Effect of nitrogen source. The mycelia of collected mushrooms were cultured in liquid medium similar to that used in the previous experiment. Nitrogen sources supplemented into the liquid medium were sodium nitrate, peptone, yeast extract, and urea [23]. Enzyme activities were measured as described previously [20, 21].

Data analysis. Results of each experiment were subjected to analysis of variance using the general linear model in SAS (SAS, Inc., Cary, NC, USA). Significance was determined by Duncan's multiple range test at the 0.5% probability level.

Results and Discussion

Influence of pH. Five different pH (3.8, 4.8, 5.8, 6.8, and 7.8), with three replicates of each treatment, were used for the ten mushroom samples investigated (Table 1). Generally, the mushrooms had their highest amylase and cellulase values at a pH range of 5.8~6.8. These values fell within the range reported by Jahangeer *et al.* [21] for different fungal isolates. No or low enzyme activity was demonstrated at pH 3.8, which may have been attributed to toxicity and consequent damage inflicted on the cell walls and membranes at a highly acidic pH. Griffin [24] and Garraway and Evans [25] independently suggested that cell membrane permeability may be adversely affected at highly acidic pH. Jonathan [2] reported that a pH of 4.0 (and below) resulted in negative effects on cell membrane regulatory function, in the passage of essential nutrients

Table 1. Effect of pH on amylase and cellulase activities (unit/mL) of wild fungi

Organisms	pH									
	3.8		4.8		5.8		6.8		7.8	
	AML	CEL	AML	CEL	AML	CEL	AML	CEL	AML	CEL
<i>Pogonomyces hydroides</i>	0.09d	0.07bc	0.15f	0.11cd	0.19ef	0.24de	0.19e	0.27e	0.15e	0.19d
<i>Termitomyces clypeatus</i>	0.24b	0.09b	0.20de	0.20bc	0.23de	0.56abc	0.23d	0.56abc	0.17e	0.48b
<i>Nothopanus hygrophanus</i>	0.08d	0.02cd	0.15f	0.06dc	0.16f	0.18e	0.15f	0.16e	0.11e	0.12e
<i>Podoscypha bolleana</i>	0.13cd	0.16a	0.16f	0.12cd	0.21de	0.22de	0.22de	0.22e	0.18e	0.14de
<i>Corilopsis occidentalis</i>	0.31a	-----	0.21d	0.25ab	0.31c	0.52b	0.29c	0.53bc	0.27cd	0.47b
<i>Agaricus blazei</i>	0.16c	-----	0.37a	0.14cd	0.59a	0.16e	0.60a	0.24e	0.48a	0.13de
<i>Termitomyces globulus</i>	0.12cd	0.10b	0.24d	0.34a	0.24d	0.31cd	0.25cd	0.40d	0.25d	0.49b
<i>Coriolus versicolor</i>	0.16c	0.06bc	0.18ef	0.32a	0.29c	0.62ab	0.28c	0.67a	0.28c	0.54ab
<i>Pleurotus tuber-regium</i>	0.10cd	-----	0.33b	0.20bc	0.40b	0.63a	0.42b	0.64ab	0.38b	0.56a
<i>Agaricus</i> sp.	0.13cd	0.08b	0.28c	0.14cd	0.39b	0.40c	0.39b	0.51cd	0.32bc	0.40c

Values with the same letter(s) in each column are not significantly different (Duncan's multiple range test). Each value is an average of three replicates ($p \leq 0.05$).

AML, amylase; CEL, cellulase.

needed for enzyme action, the metabolic role and vegetative growth of some Nigerian higher fungi.

The highest cellulase activity (0.67 unit/mL) was recorded in *Coriolus versicolor* at a pH of 6.8 followed in order ($p \geq 0.05$) by *P. tuber-regium*, *T. clypeatus* and *C. occidentalis*. Apart from *N. hygrophanus* and *P. bolleana*, which showed 0.18 and 0.22 unit/mL of cellulase activity at pH 5.5, all other fungi had their highest cellulase activities at a slightly acidic pH of 6.8 (Table 1). The lowest cellulase activity (0.02 units/mL) was observed for *N. hygrophanus* at pH 3.8 and *C. occidentalis*, *A. blazei* and *P. tuber-regium* demonstrated no enzyme activity at pH 3.8. *Pleurotus tuber-regium* and *Agaricus* sp. had the highest amylolytic activity values of 0.6, 0.42, and 0.39 unit/mL, respectively, at a pH of 6.8, whereas *P. hydroides* and *N. hygrophanus* had the lowest values of 0.09 and 0.08 unit/mL, respectively, at pH 3.8 (Table 1). The

reasons for the better cellulase than amylase activity may be attributed to the habitat and the substrates where the mushrooms grow.

Influence of temperature. The influence of temperature on enzyme action in the mushrooms is presented in Table 2. The highest amylase and cellulase activities were noted between 25 and 30°C. At 25°C, *C. versicolor* demonstrated the highest cellulase activity of 0.67 unit/mL, followed by *P. tuber-regium* and *T. clypeatus* with 0.64 and 0.56 unit/mL, respectively. Other mushrooms that had very high cellulase activity at 25°C were *Pogonomyces hydroides*, *Podoscypha bolleana*, *A. blazei*, and *Agaricus* sp. Mushrooms that had their maximum cellulase action at 30°C were *N. hygrophanus*, *P. bolleana*, *C. occidentalis*, *T. globulus*, and *P. tuber-regium*. A similar effect of temperature on laccase production was reported in *Schizo-*

Table 2. Effect of temperature on amylase and cellulase activities (unit/mL) of wild fungi

Organisms	Temperature (°C)							
	25		30		35		40	
	AML	CEL	AML	CEL	AML	CEL	AML	CEL
<i>Pogonomyces hydroides</i>	0.19e	0.27e	0.19ef	0.17fg	0.10c	0.08d	0.06b	0.09bcd
<i>Termitomyces clypeatus</i>	0.23d	0.56abc	0.25de	0.46cd	0.22b	0.11cd	0.11ab	0.07d
<i>Nothopanus hygrophanus</i>	0.15f	0.16e	0.16f	0.26e	0.07c	0.12cd	0.06b	0.01e
<i>Podoscypha bolleana</i>	0.22de	0.22e	0.24de	0.22ef	0.20b	0.09d	0.08b	-----
<i>Corilopsis occidentalis</i>	0.29c	0.53bc	0.29cd	0.55ab	0.23b	0.06d	0.08b	0.1bc
<i>Agaricus blazei</i>	0.60a	0.24e	0.52a	0.11g	0.09c	0.18c	0.11ab	0.08bcd
<i>Termitomyces globules</i>	0.25cd	0.40d	0.23de	0.58ab	0.23b	0.34b	0.16a	0.06cd
<i>Coriolus versicolor</i>	0.28c	0.67a	0.34c	0.53bc	0.22b	0.62a	0.08b	0.09bcd
<i>Pleurotus tuber-regium</i>	0.42b	0.64ab	0.41b	0.64a	0.34a	0.32b	0.08b	0.14a
<i>Agaricus</i> sp.	0.39b	0.51cd	0.35bc	0.41d	0.26b	0.09d	0.07b	0.11ab

Values with the same letter(s) in each column are not significantly different (Duncan's multiple range test). Each value is an average of three replicates ($p \leq 0.05$).

AML, amylase; CEL, cellulase.

phyllum commune by Adejoye and Fasidi [26]. Gbolagade *et al.* [6] also obtained very good biomass yield within the temperature range of 25–30°C. The high cellulolytic activities in *C. versicolor* and *P. tuber-regium* may be associated with their natural habitats. Both fungi are wood degrading fungi [27].

Very good amylase activities (0.60 and 0.24 unit/mL) were recorded for *A. blazei* and *Podoscypha bolleana* at 25 and 30°C, respectively (Table 2). Conversely, little or no amylase and cellulase activity were observed at 35 and 40°C. Enzyme activity is reduced at high temperatures [24]. Table 2 results are similar to those obtained by Jayasinghe *et al.* [27], who suggested that 25–30°C was a favourable temperature for mycelial growth of a wild strain of *Ganoderma lucidum* from Korea. Similarly, Adejoye and Fasidi [26] reported that 28°C was the optimum temperature for producing laccase in *S. commune*. However, Fasidi [4] reported that 35°C is an ideal temperature for mycelial yield of *V. esculenta*.

Carbohydrate sources. All four carbon compounds (CMC, glucose, maltose, and sucrose) significantly enhanced amylase and cellulase production in the tested fungi (Table 3). CMC promoted the highest cellulase activities (0.67 and 0.64 unit/mL) in *C. versicolor* and *P. tuber-regium*, respectively. CMC also stimulated very good amylase activity (0.60 unit/mL) in *A. blazei*. The high performance of these two fungi in CMC may be due to the availability of cellulase, which catalyses the hydrolysis of cellulose to assimilable simple sugars. This result is different from that reported by Fasidi [4] for *V. esculenta*. He found that glucose and fructose stimulated mycelial biomass production most in *V. esculenta*. This difference may be due to the genera of the mushrooms used.

Glucose also enhanced significant enzyme activity in

all tested mushrooms (Table 3). Hexose stimulated cellulase production in *C. occidentalis* (0.60 unit/mL), *Pogonomyces hydnooides* (0.25 units/mL), *N. hygrophanus* (0.24 unit/mL) and *Podoscypha bolleana* (0.22 units/mL). Similar utilization of glucose was reported by Jonathan [2] for different Nigerian higher fungi, Jayasinghe *et al.* [27] and Adejoye and Fasidi [26] reported glucose as the most stimulatory carbohydrate source for mycelial production in *G. lucidum* and *S. commune*. Glucose utilization for enzyme production may be attributed to the ease by which this sugar is oxidized to produce cellular energy. Generally, the two disaccharides (maltose and sucrose) used in this study were not supportive of insulin for enzyme production, which agreed with an earlier observation by Gbolagade *et al.* [6], who suggested that oligosaccharides must be hydrolysed to simple sugars before they can be incorporated into the fungal cell.

Nitrogen compounds. The effect of four nitrogen sources on amylase and cellulase activities in the fungi are presented in Table 4. The highest cellulase activity (0.68 units/mL) was observed in *C. versicolor* in yeast extract substituted submerged liquid medium. Other mushrooms that had very good cellulase activities with this complex nitrogen source were *P. hydnooides*, *T. clypeatus*, *T. globulus*, *P. tuber-regium*, and *Agaricus* sp. Jonathan [2] also obtained the highest mycelial biomass yield in *L. subnudus*, *P. giganteus*, and *P. atrombonata* with yeast extract. Adejoye and Fasidi [26] reported very good biomass and laccase production in *S. commune* using a yeast extract. Enhanced enzyme production by the yeast extract may also be attributed to its high vitamin and amino acid composition [14].

Urea was also stimulatory to cellulase production in *P. tuber-regium* and *N. hygrophanus* with values of 0.61 and 0.20 units/mL, respectively (Table 4). Urea also enhanced

Table 3. Effect of carbon sources on amylase and cellulase activities (unit/mL) of wild fungi

Organisms	Carbon sources							
	CMC		Glucose		Maltose		Sucrose	
	AML	CEL	AML	CEL	AML	CEL	AML	CEL
<i>Pogonomyces hydnooides</i>	0.19e	0.27e	0.18de	0.25d	0.14ef	0.21fg	0.11de	0.01de
<i>Termitomyces clypeatus</i>	0.23d	0.56abc	0.22cd	0.52d	0.24cd	0.46c	0.18c	0.23cd
<i>Nothopanus hygrophanus</i>	0.15f	0.16e	0.08f	0.24d	0.09f	0.24ef	0.09e	-----
<i>Podoscypha bolleana</i>	0.22de	0.22e	0.14ef	0.22de	0.15ef	0.19g	0.13de	0.22cd
<i>Corilopsis occidentalis</i>	0.29c	0.53bc	0.19cde	0.60a	0.19cde	0.57a	0.15cd	0.37ab
<i>Agaricus blazei</i>	0.60a	0.24e	0.41a	0.18e	0.19b	0.26e	0.24b	0.01e
<i>Termitomyces globulus</i>	0.25cd	0.40d	0.21cd	0.26d	0.17de	0.28e	0.18c	0.19cd
<i>Coriolus versicolor</i>	0.28c	0.67a	0.24c	0.56ab	0.24c	0.54b	0.32a	0.37a
<i>Pleurotus tuber-regium</i>	0.42b	0.64ab	0.44a	0.56ab	0.40a	0.52b	0.26b	0.25bc
<i>Agaricus</i> sp.	0.39b	0.51cd	0.33b	0.36c	0.21cde	0.37d	0.15cd	0.14cd

Values with the same letter(s) in each column are not significantly different (Duncan's multiple range test). Each value is an average of three replicates ($p \leq 0.05$).

AML, amylase; CEL, cellulase.

Table 4. Effect of nitrogen sources on amylase and cellulase activities (unit/mL) of wild fungi

Organisms	Nitrogen sources							
	Urea		Peptone		Yeast extract		NaNO ₃	
	AML	CEL	AML	CEL	AML	CEL	AML	CEL
<i>Pogonomyces hydroides</i>	0.20cde	0.22d	0.18cd	0.15e	0.19de	0.24d	0.12c	0.08de
<i>Termitomyces clypeatus</i>	0.24cde	0.50b	0.24bc	0.45c	0.22d	0.51b	0.20b	0.15c
<i>Nothopanus hygrophanus</i>	0.13d	0.20d	0.11d	0.16e	0.15f	0.14d	0.08c	0.07de
<i>Podoscypha bolleana</i>	0.18cd	0.19d	0.19cd	0.10e	0.19ef	0.14d	0.11c	0.15e
<i>Corilopsis occidentalis</i>	0.30bcd	0.20d	0.29ab	0.12e	0.27c	0.16d	0.23b	0.06e
<i>Agaricus blazei</i>	0.30bc	0.23d	0.25bc	0.24d	0.18ef	0.19d	0.21b	0.12cd
<i>Termitomyces globulus</i>	0.27bcd	0.31c	0.29ab	0.27d	0.28c	0.35c	0.33a	0.24b
<i>Coriolus versicolor</i>	0.28bcd	0.66a	0.36ab	0.74a	0.32b	0.68a	0.32a	0.14a
<i>Pleurotus tuber-regium</i>	0.43a	0.61a	0.37ab	0.65b	0.36a	0.59b	0.29a	0.43a
<i>Agaricus sp.</i>	0.37ab	0.51b	0.38a		0.37a	0.56b	0.23b	0.26b

Values with the same letter(s) in each column are not significantly different (Duncan's multiple range test). Each value is an average of three replicates ($p \leq 0.05$).

AML, amylase; CEL, cellulase.

amylase activities in *P. tuber-regium*, *Agaricus sp.*, *C. occidentalis*, and *A. blazei* of 0.43, 0.37, 0.30, and 0.30 unit/mL, respectively. This was different from that reported by Gbolagade [28], who found a very low biomass yield of *Lepiota procera* using urea. The difference observed here may be attributed to differences in the nutrient requirements of various fungi. Peptone was also supportive of moderate amylase activity (0.19 unit/mL) in *P. bolleana*. Similar stimulation of mycelial biomass by peptone was reported by Gbolagade [28] for *L. procera*. Sodium nitrate (an inorganic nitrogen source) enhanced low enzyme activity in all mushrooms assayed. Gbolagade *et al.* [6] reported that biomass production in *Pleurotus florida* was enhanced by organic nitrogen sources, whereas inorganic nitrogen compounds generally inhibited growth of this edible fungus.

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