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Comparative studies on enzyme activities of bacterium (*Bacillus subtilis*) and Nigerian edible fungi (*Termitomyces clypeatus, Termitomyces globulus, Pleurotus tuber-regium,* and *Agaricus sp*)

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ABSTRACT

Enzyme activities of Termitomyces clypeatus, Termitomyces globulus, Pleurotus tuber-regium, and Agaricus sp (edible Nigerian fungi) were compared with that of Bacillus subtilis (a bacterium) which have been known to be a very good amylase producer. In these studies, Pleurotus tuber-regium and Agaricus sp had amylase activities values of 0.45 and 0.39 U/min respectively. Bacillus subtilis had highest amylase value with yeast extract (0.63 U/min) and least value with $NaNO_3$ (0.35unit/min). For cellulase activity, Bacillus subtilis had value of 0.4unit/min with yeast extract while Coriolus vesicolor, Pleurotus tuber-regium and Termitomyces clypetus had values of 0.67,0.59 and 0.51 U/min respectively. Temperature and pH also had significant effect on the production of these enzymes.

KEYWORDS

Amylase;
Cellulase;
Bacillus subtilis;
Mushrooms;
pH;
Temperature.

INTRODUCTION

Higher fungi are group of organisms, growing on dead organic matter which may be leaf litters, wood, richly loamy soil and other agro-industrial wastes[1-3]. These group of organisms may include mushrooms, puffballs, truffles and polypores[4,5]. Descriptions of processing methods for preparing industrial microbial enzymes have been published[5,6]. For fungal and bacterial enzymes, modifications of chemically submerged media by different authors have usually been employed for enzyme production in biological laboratories. Materials such as mineral salts, organic acid or buffer may be added to regulate the pH of the assaying medium[4,7]. Different strains of Bacillus subtilis in different media have been employed in the enzyme production[6-8]. The submerged method was originally developed and first extensively employed for production of penicillin and other antibiotics[6].

In the laboratory, submerged cultures are grown in shake Erlenmeyer flasks or in aerated tubes or Erlenmeyer flasks while for commercially, deep tanks are employed which have provision for introduction of sterile air and for uniform agitation[9-11]. Lignocellulose is main source of natural cellulose generated in agriculture, lumber, food processing and municipal service[1,15]. Microbial enzymes have been employed in food, pharmaceutical, textile, paper, leather, and other related industries and in biodegradation of cellulolytic materials[12,13].

In the studies comparism was made between enzyme production in selected Nigerian mushrooms and that of Bacillus subtilis

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MATERIALS AND METHODS

Enzyme assay

Fruitbodies of collected mushrooms (Termitomyces clypeatus, Termitomyces globulus, Pleurotus tuber-regium, and Agaricus sp) were tissue cultured and the mycelia generated were grown on Potato dextrose agar (PDA) plates for 6 days at 30±2^{0}C[5]. Hyphae of each fungus was further sub-cultured on starch-yeast–extract-agar (SYEA)[4]. Each filtrate of each the test higher fungus was assayed for amylase and cellulase enzyme using Dinitrosalicylic acid reagent[4,5]. Bacillus subtilis was grown for 24 hours on nutrient agar and the method of Ekunsanmi[12] was used for the enzyme assay of bacterium. The amount of reducing sugar that was released was determined by adding 1ml of dinitrosalicylic acid (DNSA) reagent to 1ml of filtrate-starch-reaction mixture, and the absorbance was read at 540nm using spectrophotometer.

Determination of physico-chemical parameters

Effect of pH, temperature, carbon and nitrogen compounds on amylase and cellulase activities of bacterium and fungi were determined in chemically defined basal medium[9]. The carbon sources used include soluble starch, glucose, maltose, and sucrose while the nitrogen sources used include urea, yeast extract, peptone, and $NaNO_3$. They were supplemented into the medium using the procedures described by Jonathan and Fasidi[10]. Each treatment was replicated thrice and pH was adjusted by using 0.1N HCl and 0.1N NaOH to 3.8, 4.8, 5.8, 6.8. Thirty milliliters (30ml) of each medium were dispensed into 100ml Erlenmeyer flask and sterilized; this was followed by inoculation with one 7mm alga disc plug of the macrofungus and incubated at different temperatures (28, 32, 36, and 40^{0}C respectively) for 7 days and Bacillus subtilis was grown for 48 hours after inoculation in nutrient broth. The method of Ekunsanmi[12] was used for the determination of enzyme activities in bacterium and fungi

Analysis of data

Results of each experiment was subjected to analysis of variance (ANOVA) using general linear model option SAS. Test of significance was determined by Duncan´s multiple range test at 0.5% level of probability.

RESULTS AND DISCUSSION

The study showed the amylolytic activities of various edible fungi and that of Bacillus subtilis under different assay conditions (Figure 1-8). Bacillus subtilis had high amylolytic activity value (0.60 U/min) while mushrooms such as Pleurotus tuber-regium, Agaricus sp had amylolytic activity values of 0.45 and 0.39 U/min respectively (Figure 1). It was generally observed that Bacillus subtilis demonstrated higher amylase activities than all other screened edible fungi. This result is in line with the earlier findings of Femi Ola and Aderibigbe[8] that Bacillus subtilis is a good amylase producer.

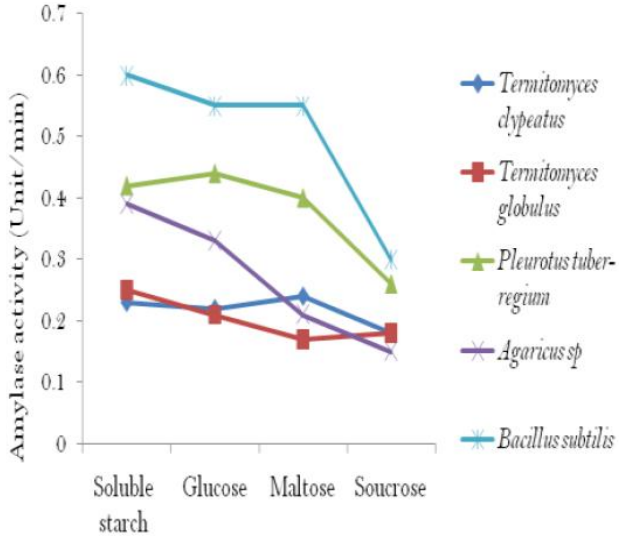

Figure 1 : Effect of carbon sources on amylase activity

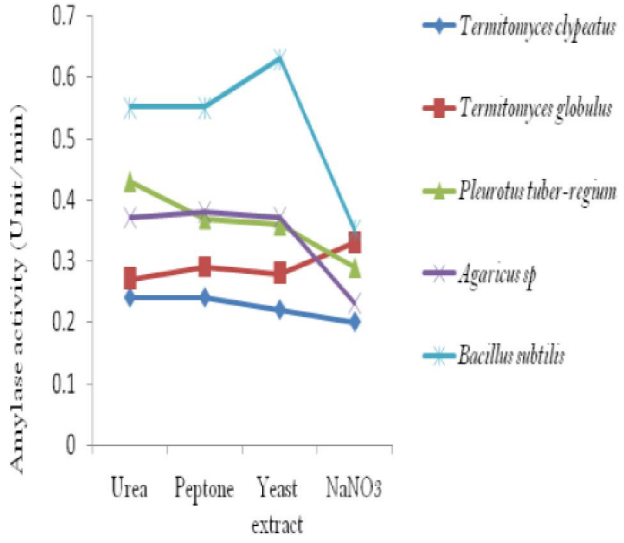

Figure 2 : Effect of nitrogen sources on amylase activity

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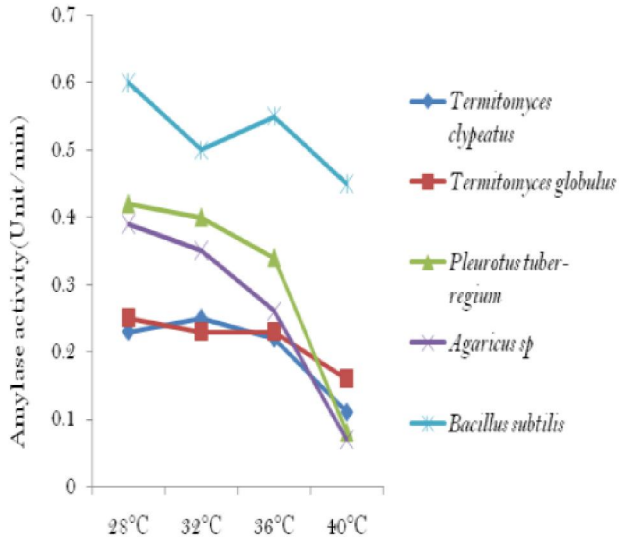

Figure 3 : Effect of temperature on amylase activity

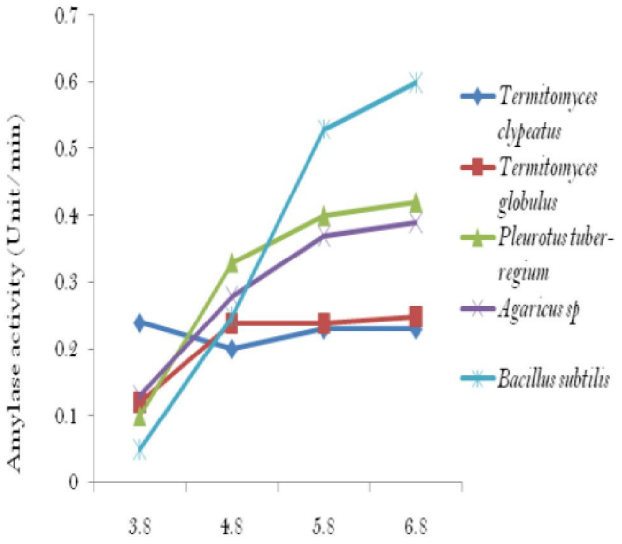

Figure 4 : Effect of pH on amylase activity

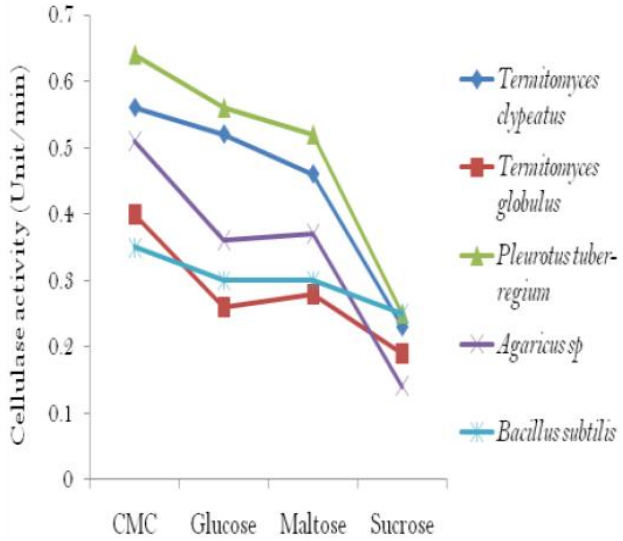

Figure 5 : Effect of carbon sources on cellulase activity

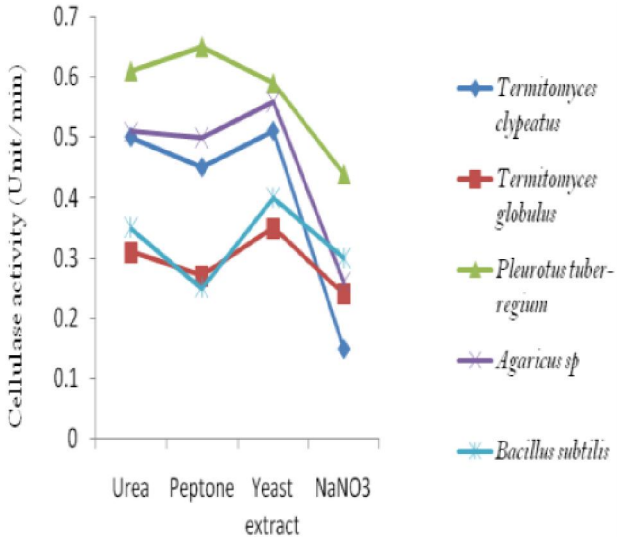

Figure 6 : Effect of nitrogen sources on cellulase activity

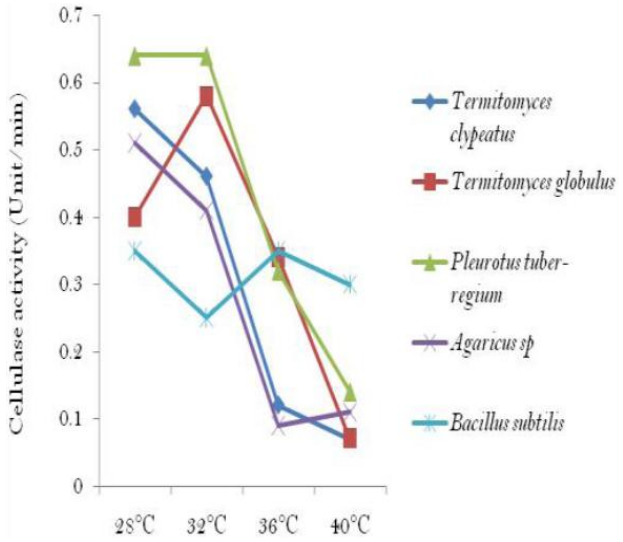

Figure 7 : Effect of temperature on cellulase activity

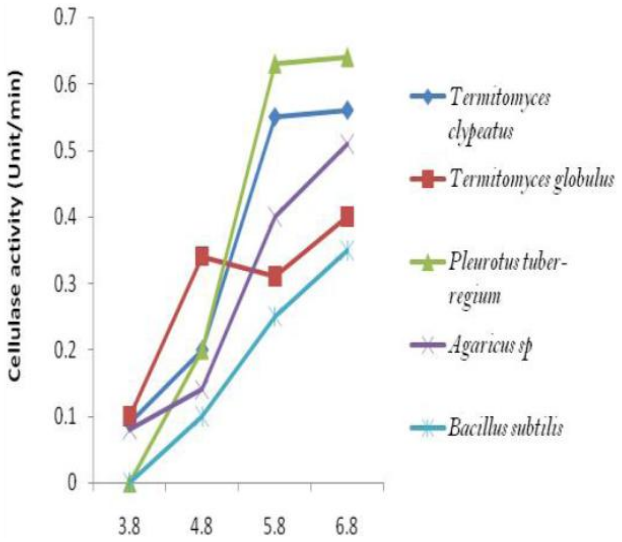

Figure 8 : Effect of pH on cellulase activity

Figure 2 shows that the test higher fungi and Bacillus subtilis utilized most of nitrogen source (urea, peptone, and yeast extract) very actively but not for $NaNO_3$ that had the least values. This observation is similar with the reports of Jonathan and Adejoye[5] and, Adjoye and Fasidi[15] that mushrooms had greater preference for organic nitrogen compounds in comparism with the inorganic nitrogen sources. Bacillus subtilis had highest amylolytic value with yeast extract (0.63 U/min) and least value with $NaNO_3$ (0.35U/min). Pleurotus tuber-regium had the highest value with urea (0.45 unit/min).

For cellulase activities, Bacillus subtilis generally had low cellulolytic activity as compared to the studied higher fungi that had higher cellulolytic activities. This may be related to the fact the most fungi studied usually grow on wood and they required the enzyme (cellulase) to degrade cellulose in the wood environment where they grow[13-15]. Pleurotus tuber-regium, Termitomyces globulus, Termitomyces clypeatus, and Agaricus sp had celululose values of 0.65, 0.58, 0.56, and 0.56 U/min respectively. The Cellulolytic activity of Bacillus subtilis was 0.4U/min with yeast extract . The higher fungi produced better cellulase than B.subtilis.

Figure 7 shows that macrofungi and Bacillus subtilis had optimal cellulolytic activity (0.35 U/min) at temperature of 28°C and least value were obtained at with 40^0C (0.3U/min). Coriolus versicolor had highest cellulolytic activity (0.67U/min) a 28°C. Bacillus subtilis and Termitomyces globulus were able to have relatively moderate cellulolytic activity (0.35U/min) at temperature of 36^0C but greater enzyme production were observed at 29-32 °C. The effect of hydrogen ion concentration (pH)on the enzyme activities of both fungi and bacterium were presented on figures 4 and 8. Generally, all the test organisms had their optimal enzyme values at pH5.8-6.8. This values fall within the range reported by Jahangeer et al[13] for fungi and, Femi Ola and Aderibigbe[8] for bacteria. Jonathan and Fasidi[10] and Parra et al[16] suggested that temperature and pH are two cardinal factors that affect metabolism of microorganisms.

CONCLUSION

The study shows that mushrooms were able to compete with Bacillus subtilis for high enzyme activities by their ability to have considerable amount ofcellulase and amylase activities. This further strengthens the fact that mushrooms and Bacillus subtilis can be employed in the production of enzymes for commercial purposes.

ACKNOWLEDGEMENTS

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