ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 08 | August - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

PRODUCTION OF LIGNOCELLULOLYTIC ENZYMES BY WILD AGARICS ISOLATED FROM LIGNOCELLULOSIC WASTES

Botany

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ABSTRACT

Background: Lignocellulosic wastes are natural, abundant and renewable resources essential to the functioning of industrial societies and critical to the development of a sustainable global economy. They are plant biomass consisting of cellulose, hemicelluloses, pectin and lignin. Lignocellulosic wastes are subjected to fungal attacks that cause deterioration by way of decomposition. Among different groups of fungi, Agarics have an edge over others in initiating the breakdown of Lignocellulosic substrates because of their superior enzymatic equipment. Agaricus compestris, Agaricus bisporus, Coprinus comatus, Lepiota sp., Marasmius sp., Tricholoma sp. Amanita muscaria, A. phalloides, A. verna etc. are common Agarics growing on lingo-cellulosic substrates. **Aim:** The aim of this study is to isolate and characterize the production of lignocellulosic enzymes different species of Agarics from different Lignocellulosic wastes. **METHODS:** Agarics were collected from lingo-cellulosic waste materials and identified on the basis of morphological features. They were cultivated on Potato Dextrose Agar (PDA), Yeast Potato Dextrose Agar (YPDA), Malt extract agar, complete Yeast extract broth and Rice bran decoction media. The linear growth characteristics and biomass production of wild agarics were studied on Complete Yeast Extract Agar Medium (CYEM). The enzyme activity (Exoglucanase, Endoglucanase and Xylanase activities of the agarics was assayed by growing the mycelia in mushroom minimal medium broth (MMMB). **Results:** Thirteen species of wild agarics were collected from lingo cellulosic substrates and taxonomically enumerated. These included Agaricus compestris, A. bisporus, A. silvicatus, A. arvensis, Coprinus comatus, Lepiota, Marasmius, Tricholoma, Amanita muscaria, A. phalloides, A. verna, Chlorophyllum molybdites and Polyporus. The linear growth of agarics was maximum for Agaricus compestris, A. bisporus, A. silvicatus, A. arvensis, Coprinus comatus, Chlorophyllum molybdites and Polyporus. Lepiota, Marasmius, Tricholoma, Amanita muscaria, A. phalloides and A. verna showed only mild to moderate linear growth of colony. The Exoglucanase and Xylanase production by Agaricus compestris, A. bisporus, A. sylvicatus, A. arvensis, Coprinus comatus and Polyporus was maximum. The endoglucanase production by Agaricus compestris, A. bisporus, A. sylvicatus, A. arvensis, Coprinus comatus and Chlorophyllum molybdites was moderate. Lepiota, Marasmius, Trocholoma, Amanita muscaria, A. phalloides and A. verna produced relatively lesser amount of Exoglucanase, endoglucanase, xylanase and laccase. **Conclusions:** All the thirteen species of agarics showed lignocellulolytic activities.

KEYWORDS

Ligno-cellulosic substrate, Agarics, Growth characteristics, Exo-glucanase, Endo-glucanase, Xylanase, Laccase.

INTRODUCTION

Lignocellulosic wastes are natural, abundant and renewable resources essential to the functioning of industrial societies and critical to the development of a sustainable global economy. They are plant biomass consisting of cellulose, hemicelluloses, pectin and lignin. The carbohydrate polymers (cellulose and hemicelluloses) are tightly bound to the lignin. Lignocellulosic biomass can be grouped into four main categories: Agricultural residues (including corn stoves and sugarcane bagasses), Dedicated energy crops, Wood residues (including saw mill and paper mill discards), and Municipal paper waste. As wood and paper products, they have played an important role in the evolution of civilization. Improvement of the quality and manufacturing efficiency of such products has often been hampered by the lack of understanding of the complex structures and chemical composition of the materials.

The Lignocellulosic biomass is basically composed of cellulose, hemicelluloses, pectin and lignin. Cellulose is a polysaccharide composed of linear glucan chains that are linked together by β-1,4-glycosidic linkages with cellobiose residues as the repeating unit at different degree of polymerization, and packed into microfibrils which are held together by intramolecular hydrogen bonds as well as intermolecular van der waals forces. Hemicellulose is the second most important biopolymer. Xylan is the main type of hemicelluloses. It is a branched heteropolysaccharide consisting of β-1,4-linked xylose units with side branches of α-arabinofuranose, α-glucuronic acid or other monosaccharide. Pectin is a structural acidic heteropolysachharide consisting of galacturonic acids joined together by α-1, 4-glycosidic linkages. Similarly, Lignin is the most abundant and complex biopolymer composed of phynylpropane units, which is synthesized by radical polymerization of guaiacyl units (G), syringyl units (S), and p-hydroxyphenyl units (H) from precursor coniferyl, sinapyle and p-coumaryl alcohol. Lignin consists of high molecular weight aromatic polymer, containing several biologically stable ether or ester linkages.

Lignocellulosic wastes are subjected to fungal attacks that cause deterioration by way of decomposition. Among different groups of fungi, Agarics have an edge over others in initiating the breakdown of Lignocellulosic substrates because of their superior enzymatic equipment. Agarics, commonly known as gill-fungi, mushrooms or toad stools, produce conspicuous basidiocarps (the fruiting bodies). Agarics may be edible, poisonous or unpalatable. Edible agarics are commonly known as mushrooms, and poisonous ones, the 'toad stools'. They are mostly saprophytic fungi growing commonly in lawns, pasture and gardens. Agaricus compestris, Agaricus bisporus, Coprinus comatus, Lepiota sp., Marasmius sp., Tricholoma sp. etc. are edible mushrooms. The poisonous agarics include Amanita muscaria, A. phalloides, A. verna etc.

The climatic condition of Patna is humid being the most favorable for the growth of agarics and other macro and micro fungi. 650 species of Agarics, 159 species of Polyporus, 59 species of Fomes, 35 species of Trametes, 27 species of Poria, 55 taxa of Russula and 26 taxa of Lactaricus have been reported from India (Manohararachary et al., 2005)[1].

Agarics growing on lignocellulosic wastes have been studied by several mycologists viz. Mehdi Dustban et al., (2009)[2], A. J. Durantd et al., (1991)[3], Ramendra Kumar Sinha et al., (2023)[4], Abd-Elsalan and Hanofy (2009)[5], Alessi et al., (2018)[6], Cortes-Tolapa et al., (2017, 2018)[7,8] etc. The production of lignocellulolytic enzymes from Agarics growing on lignocellulosic wastes in Patna zone has not been studied so far. Hence the present study has been undertaken. The aim of this study is to isolate and characterize different species of Agarics from different Lignocellulosic wastes of Patna.

MATERIALS AND METHODS

Wild Agarics were collected from lignocellulosic wastes viz. rotten woods, bagasse fibers, dung, corn stoves during rainy season. Agarics were dig out carefully and put into the sterilized polythene bag and brought to laboratory for identification and characterization.

Uncapped mushrooms were preferred and collected in triplicate forms.

The agarics collected from lingo-cellulosic wastes were cultivated and cultured in laboratory by producing their spawn. Spawn is the pure culture of the mycelium grown on a special medium. Potato Dextrose Agar (PDA), Yeast Potato Dextrose Agar (YPDA), Malt extract agar, complete Yeast extract broth and Rice bran decoction media were used for maintenance, multiplication and preservation of agarics.

The production of spawn consisted of three steps viz. preparation of substrate, inoculation and incubation. The substrate for spawn production was wheat grain. 500 gm of wheat grain was boiled in 500 mL of water for 20 minutes. Excess water was removed and the grains were dried by spreading over alkaline sheets in shade for a few hours. The grains were then mixed with calcium sulphate and calcium carbonate at 2% and 5% respectively on dry weight basis of the grain. pH was adjusted to 7.8. The grain-chemical mixture was filled in 500 mL flasks. The flasks were plugged with non-absorbent cotton and sterilized at 15lbpressure (121^0C) in an autoclave. The substrate was then inoculated with the mycelium of agarics grown on PDA medium under aseptic condition and incubated at 25^0C for three weeks in darkness.

The linear growth characteristics and biomass production of wild agarics were studied on Complete Yeast Extract Agar Medium (CYEM) after 10 days of incubation. Biomass production ability of each of the wild agarics was observed in Complete Yeast extract broth (CYEB). A mycelial bit of 5 mm diameter was inoculated in 50 mL of complete yeast extract broth and the biomass was measured after 5 and 10 days of incubation. The mycelia were dried at 55^0C for 4 hours and the biomass was measured by subtracting out the dry weight of filter paper.

The enzyme activity of the wild agarics was assayed by growing the mycelia in mushroom minimal medium broth (MMMB). The MMMB was inoculated with wild agarics growing on the master plates and incubated at 30^0C for 10 days in BOD incubator. The culture was filtered through Whatman filter paper number-1 after 5 and 10 days of incubation. The culture filtrate was stored at 4^0C. The exoglucanase, endoglucanase and xylanase activity were assayed by method as suggested by Sandhu and Kalra (1982)[9]. Laccase activity was assayed by method as suggested by Dhaliwal et al., (1991)[10]. For exoglucanase, endoglucanase and xylanase the absorbance of enzyme was recorded at 540 nm, and for laccase the absorbance of enzyme was recorded at 495 nm using UV-Vis spectrophotometer. The amount of reducing sugar released was estimated using glucose standard curve. The total protein was estimated by method as suggested by Lowry (1951)[11]. The specific enzyme activity was calculated by following formula:

$$\text{Specific enzyme Activity in U/mg} = \frac{\text{Relative Activity in U/mL}}{\text{Protein concentration in mg/mL}}$$

All experiments were carried out in replicates of three and the data was analyzed by measuring standard deviation (SD), standard error (SE) and critical difference (CD) at 5% level of significance.

RESULTS

In course of present investigation, thirteen species of wild agarics were collected from lingo cellulosic substrates and taxonomically enumerated. These included Agaricus compestris, A. bisporus, A. silvicatus, A. arvensis, Coprinus comatus, Lepiota, Marasmius, Tricholoma, Amanita muscaria, A. phalloides, A. verna, Chlorophyllum molybdites and Polyporus.

Growth Characteristics of Agarics

Growth characteristics of all the thirteen agarics were studied in vitro on complete yeast extract medium after 5, 10, 15, 20 and 25 days of incubation. Petri dishes used were 90 mm in diameter, and after inoculation the culture was incubated at 25 ± 2^0C in an incubator. pH of the medium was adjusted at 6.5. The results obtained have been presented in Table-1.

Table-1: In Vitro Growth Characteristics of Agarics

Agarics	Linear growth of colony in mm				
	Days of incubation				
	5	10	15	20	25
Agaricus compestris	7.5 ±0.45	9.7 ±0.35	14.8 ±0.26	17.6 ±0.35	18.5 ±0.46
Agaricus bisporus	7.6 ±0.44	9.5 ±0.32	14.5 ±0.26	16.8 ±0.24	18.4 ±0.45
Agaricus silvicatus	6.7 ±0.31	9.0 ±0.22	12.5 ±0.42	15.5 ±0.55	17.6 ±0.36
Agaricus arvensis	6.3 ±0.25	8.5 ±0.27	10.5 ±0.24	13.6 ±0.41	15.5 ±0.27
Coprinus comatus	6.8 ±0.35	8.7 ±0.36	11.6 ±0.48	15.8 ±0.51	18.5 ±0.33
Lepiota	5.2 ±0.21	7.8 ±0.27	9.6 ±0.44	13.7 ±0.34	15.7 ±0.35
Marasmius	5.0 ±0.27	6.8 ±0.26	9.0 ±0.52	13.0 ±0.27	14.8 ±0.45
Tricholoma	4.7 ±0.22	7.0 ±0.25	8.8 ±0.41	11.5 ±0.28	14.7 ±0.34
Amanita muscaria	5.3 ±0.17	7.5 ±0.11	9.6 ±0.18	13.7 ±0.36	15.7 ±0.31
Amanita phalloides	5.5 ±0.19	8.2 ±0.16	10.2 ±0.42	14.5 ±0.37	17.6 ±0.35
Amanita verna	6.0 ±0.12	8.3 ±0.17	11.5 ±0.25	16.0 ±0.30	18.5 ±0.24
Chlorophyllum molybdites	7.5 ±0.27	9.5 ±0.41	15.5 ±0.27	18.6 ±0.28	19.5 ±0.26
Polyporus	7.0 ±0.25	9.8 ±0.27	15.7 ±0.36	18.8 ±0.37	19.7 ±0.28

The in vitro growth of agarics was maximum for Agaricus compestris, A. bisporus, A. silvicatus, A. arvensis, Coprinus comatus, Chlorophyllum molybdites and Polyporus. After 25 days of incubation these agarics showed linear 18.5 mm, 18.4 mm, 17.6 mm, 15.5 mm, 18.5 mm, 19.5 mm and 19.7 mm of linear growth of colony. Lepiota, Marasmius, Tricholoma, Amanita muscaria, A. phalloides and A. verna showed only mild to moderate linear growth of colony (Table-1).

The biomass production of present agarics was also studied after 5, 10, 15, 20 and 25 days of incubation at temperature 25 $\pm2^0$C and pH 6.5. The results obtained have been presented in Table-2.

Table-2: Biomass Production of Agarics (Dry Weight of Mycelium in gm/day)

Agarics	Dry weight of mycelium in gm/day				
	Days of incubation				
	5	10	15	20	25
Agaricus compestris	1.24 ±0.11	2.50 ±0.17	3.75 ±0.20	4.56 ±0.21	5.75 ±0.17
Agaricus bisporus	1.25 ±0.12	2.47 ±0.16	3.65 ±0.14	4.51 ±0.15	5.70 ±0.14
Agaricus sylvicatus	1.07 ±0.08	1.75 ±0.07	2.85 ±0.13	3.65 ±0.17	4.11 ±0.13
Agaricus arvensis	1.12 ±0.12	1.65 ±0.16	2.75 ±0.15	3.45 ±0.16	4.00 ±0.12
Coprinus commatus	1.23 ±0.15	2.46 ±0.13	3.67 ±0.11	4.58 ±0.17	5.75 ±0.21
Lepiota	0.85 ±0.16	1.07 ±0.16	1.75 ±0.09	2.35 ±0.14	2.85 ±0.31
Marasmius	0.89 ±0.16	1.10 ±0.31	1.85 ±0.17	2.65 ±0.09	2.95 ±0.32
Tricholoma	0.91 ±0.11	1.15 ±0.21	2.00 ±0.20	2.75 ±0.17	3.00 ±0.15
Amanita muscaria	0.75 ±0.12	0.97 ±0.32	1.15 ±0.13	1.87 ±0.16	2.13 ±0.21
Amanita phalloides	0.76 ±0.11	0.95 ±0.16	1.13 ±0.14	1.86 ±0.20	2.11 ±0.24
Amanita verna	0.77 ±0.17	0.94 ±0.16	1.14 ±0.12	1.90 ±0.14	2.17 ±0.22
Chlorophyllum molybdites	0.85 ±0.15	9.70 ±0.13	1.17 ±0.14	1.95 ±0.08	2.23 ±0.07
Polyporus	1.16 ±0.06	2.15 ±0.08	3.16 ±0.16	3.85 ±0.15	4.16 ±0.31

Biomass production of present agarics was maximum for Agaricus compestris, A. bisporus, A. syllvicatus, A. arvensis, Coprinus comatus and Polyporus. The mcelial dry weight of these agarics was 5.70, 4.11, 4.00, 5.75 and 4.16 gm/day after 25 days of incubation. Other agarics showed only mild to moderate production of miomass (Table-2).

Enzyme Aactivity of Agarics

For enzymatic activity all the present agarics were cultured mushroom

minimal media broth for 10 days of incubation. The culture of each agarics was filtered through whatman no.1 filter paper and the filtrate was stored at 4^0C and assayed for exoglucanase, endoglecanase, xylanase and laccase activities. The results obtained have been presented in Table-3.

Table-3: Enzyme Activity of Wild Agarics

Agarics	Specific enzyme activity in U/mg protein			
	Exo-glucanase	Endo-glucanase	Xylanase	Laccase
Agaricus compestris	0.525 ±0.07	0.187 ±0.05	0.516 ±0.07	3.35 ±0.11
A. bisporus	0.526 ±0.10	0.193 ±0.12	0.519 ±0.09	3.14 ±0.14
A. sylvicatus	0.515 ±0.11	0.176 ±0.07	0.495 ±0.10	2.85 ±0.13
A. arvensis	0.516 ±0.15	0.180 ±0.10	0.512 ±0.15	3.26 ±0.17
Coprinus commatus	0.517 ±0.16	0.190 ±0.12	0.513 ±0.16	3.14 ±0.12
Lepiota	0.135 ±0.07	0.097 ±0.02	0.143 ±0.07	0.170 ±0.13
Marasmius	0.141 ±0.07	0.096 ±0.03	0.141 ±0.05	0.167 ±0.11
Tricholoma	0.136 ±0.05	0.065 ±0.01	0.147 ±0.07	0.185 ±0.13
Amanita muscaria	0.125 ±0.04	0.090 ±0.03	0.145 ±0.10	0.173 ±0.16
A. phalloides	0.127 ±0.06	0.087 ±0.03	0.146 ±0.11	0.165 ±0.15
A. verna	0.122 ±0.04	0.088 ±0.01	0.143 ±0.11	0.161 ±0.12
Chloro-phyllum molybdites	0.145 ±0.08	0.185 ±0.03	0.114 ±0.12	0.375 ±0.10
Polyporus	0.547 ±0.07	0.217 ±0.06	0.520 ±0.10	0.387 ±0.06

From the results it is evident that the Exoglucanase and Xylanase production by Agaricus compestris, A. bisporus, A. sylvicatus, A. arvensis, Coprinus comatus and Polyporus was maximum. The specific enzyme activity for Exo glucanase by these agarics was 0.525 U/mg, 0.526 U/mg, 0.515 U/mg, 0.516 U/mg, 0.517 U/mg and 0.547 U/mg of enzyme protein respectively. Similarly, the Xylanase activity was 0.516 U/mg, 0.519 U/mg, 0.495 U/mg, 0.512 U/mg, 0.513 U/mg and 0.514 U/mg of enzyme protein respectively. The endoglucanase production by Agaricus compestris, A. bisporus, A. sylvicatus, A. arvensis, Coprinus comatus, Chlorophyllum molybdites was 0.187 U/mg, 0.193 U/mg, 0.176 U/mg, 0.180 U/mg, 0.190 U/mg, 0.185 U/mg and 0.217 U/mg of enzyme protein respectively. Lepiota, Marasmius, Trocholoma, Amanita muscaria, A. phalloides and A. verna produced relatively lesser amount of Exoglucanase, endoglucanase, xylanase and laccase. Similarly, the lacase production by Agaricus compestris, A. bisporus, A. sylvestris, A. arvensis, Coprinus comatus was high about 3.35 U/mg, 3.41 U/mg, 2.85 U/mg, 3.26 U/mg, 3.41 U/mg of enzyme protein respectively (Table-3). Other agarics produced relatively lesser amount of laccase.

DISCUSSION

In the present study thirteen wild agarics were collected from lignocellulosic substrates viz. sugarcane Bagasse, woods, dung etc. These were cultivated and cultured in laboratory. Agaricus compestris, A, bisporus, A. sylvicatus, A. arvensis, Coprinus comatus, Lepiota, Marasmius, Tricholoma, Amanita muscaria, A. phalloides, A. verna, Chlorophyllum molybdites and Polyporus were characterized. Species of Agaricus showed pinkish lamellae during younger stage of basidiocarps which turned brown on maturity. The present observations gain support from the work of Karwa and Rai (2010)[12] who have also observed similar characteristics of species of Agaricus. Mamony and Saleh (2009)[13] have observed more or less similar features of Agaricus sylvicatus. Chlorophyllum molybdites showed more or less similar morphological features with Lepiota brunea and Lepiota rachodes. Similar behavior of Lepiota has also been studied by Kumar and Kaviayarasan (2012)[14]. Kang et al., (2014)[15] have also collected and characterized some members of Basidiomycetes from Ludhiana (Punjab) and found more or less similar characteristics of Basidiomycetous fungi. Other agarics showed similar pattern of growth as indicated in standard monographs.

Radial growth of mycelium of all the thirteen wild agarics was studied after 5, 10, 15, 20 and 25 days of incubation. The results revealed that the linear growth rate of Agarics increased with increase in incubation time. After 25 days of incubation maximum linear growth of mycelia was observed in the range of 14.7 mm/day to 19.7 mm/day (Table-1). More or less similar growth pattern was noticed by Shittu et al., (2005)[16], Kang et at., (2014)[15].

The biomass production in agarics increased with increase in incubation time. After 25 days of incubation the biomass production of Agaricus compestris, A.bisporus, A. sylvicatus, A. arvensis, Coprinus comatus and Polyporus was high in comparison to other agarics. These agarics showed 5.70, 4.11, 4.0, 5.75 and 4.16 gm/day of biomass production (Table-2). Hassan and Ghada (2012)[17] have also observed more or less similar biomass production in Mushrooms.

Agaricus compestris, A. bisporus, A. sylvicatus, A. arvensis, Coprinus comatus and Polyporus showed maximum exoglucanase, endoglucanase, xylanase and laccase activities (Table-3) compared to other agarics. The present findings are in accordance with the work of Savoie et al., (1996)[18] and Kang et al., (2014)[15]. However, different agarics showed great variation in their lignocellulolytic capabilities.

CONCLUSIONS

It can be concluded that all the thirteen species of agarics showed moderate to high lignocellulolytic activities. The present study also indicated a good diversity of mushrooms growing in the lingo-cellulosic substrates.

Conflict Of Interest: Authors declare no conflict of interest directly or indirectly.

Acknowledgement: Authors are thankful to Dr. Baidyanath Kumar for providing necessary suggestion.

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