



Research Article

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# Useful Extracellular Enzymatic Activity of Mycelial Culture of Some Edible Mushrooms of Odisha

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## Abstract

Screening of extracellular enzymatic activities of the seven mushroom mycelia was taken into consideration for which most popular and palatable mushroom from Odisha such as *Russula lepida*, *Russula brevipes*, *Russula nigricans*, *Volvariella volvacea*, *Lentinus tuberregium*, *Macrolepiota procera* and *Calocybe indica* were served as the model organisms. Studies in this relevance showed varying enzymatic activities, a good L-asparaginase activity was recorded in *R. nigricans* and *R. brevipes*. Mushroom mycelia of *R. nigricans* showed an appreciable amount of cellulase activity along with phosphate solubilization potentialities; best lipase activity was recorded in mycelial culture of *R. brevipes*. Almost all the studied mushroom mycelia showed positive test for the IAA production and a poor protease activity was seen in *R. brevipes* where all other species showed no proteolytic activity.

**Keywords:** Enzymes; Mushrooms; Protease; Cellulase; Amylase; IAA; Phosphate solubilization

## Introduction

Biodegradation involves microbes for the solubilization of insoluble macromolecules like keratin, cellulose, collagen, lignin, chitin and casein which depends on the secretion of extracellular enzymes in their substrates Abbas et al. [1], Bockle et al. [2], Friedrich et al. [3], Allpress et al. [4] this kind of activities shown by the microbes helps in the mineral balance in nature. In the same way term "Biodeterioration" is mainly pronounced for the undesirable activity of microbes on useful substances, which causes immense economic losses. Overall in all the conditions, microorganisms produces extracellular enzymes, which helps for hydrolyzing the complex organic substances into simpler forms that can be utilized and assimilated by them or released in surrounding free environment Rao et al. [5], Kumar and Takagi [6] Kirk et al. [7] Nehra [8], Amoozegara et al. [9]. The production of useful enzymes by plant and animal sources such as proteases, cellulase, Xylanase and lipase are not sufficient to meet the current industrial demands which has drawn upon the interest of researchers towards microbial enzymes, since they have been known to possess almost all features desired for useful biotechnological as well as other industrial applications Forgatty and Kelly, [10] Singh [11] Beg et al. [12], Ellaiah et al. [13]

Nascimento et al. [14], Gouda et al. [15]. Ectomycorrhizal fungi show varying phosphatase activity between species, resulting in different efficiency of phosphate solubilization of host plant Ho and Zak [16]. Moreover factors like edaphic components, pH and mineral constituents can modify the conformation of enzymes and affect their activities Eivazi and Tabatabai [17].

Mushrooms are the known for the various pharmaceutical, nutraceutical and extracellular enzyme productions; however reports regarding certain enzymes like IAA production and extracellular organic acid production are very less. Some reports suggests Proteolytic activity Nakamura et al. [18], Terashita et al. [19], Nonaka et al. [20], Healy et al. [21], Cellulytic activity Kumaran et al. [22], Madan and Bisara [23], amylase activity Jonathan and Adeoyo [24], Xylanase activity Ghosh et al. [25], Lee et al. [26], L-Asparaginase activity Eisele et al. [27], Mishra [28], Lipase Shu et al. [29], IAA production Bose et al. [30], Phosphate solubilising activity Lapeyrie et al. [31], Leyval and Berthelin [32] from macrofungal sources. Present study was intended to screen the extracellular production enzymes, organic acids, IAA and phosphate solubilization activity from the mushroom mycelium under plate culture conditions (Table 1).

**Table 1:** Extracellular enzymatic activities of mycelium of some common edible mushrooms in Odisha.

S. no	Species	Amylase	Protease	Lipase	Xylanase	Cellulase	L-Asparaginase	Organic acid production	IAA	Phosphate solublizing
1	<i>R. lepida</i>	-	-	-	+	+	++	-	+	-
2	<i>R. nigricans</i>	-	-	-	-	+++	+++	+++	++	++
3	<i>R. brevipes</i>	+	+	++++	-	++	+++	-	+	-
5	<i>V. volvaceae</i>	-	-	-	-	-	-	-	-	-
6	<i>L. tuberregium</i>	-	-	+++	-	-	+	-	+++	-
7	<i>C. indica</i>	-	-	-	-	+	+	-	++	-

## Materials and Methods

### Collection and identification

Mushrooms species of *R. lepida*, *R. brevipes*, *R. nigricans*, *M. procera*, *V. volvaceae*, *L. tuberregium* and *C. indica* were collected from tropical moist deciduous and semi ever green forest of Odisha (India). For identification, macroscopic and microscopic examination of pileus, stipe, veil, ring, volva, lamellae and gills etc. were taken into consideration according to Largent [33].

### Preparation of master culture plate

Wild edible mushrooms were collected from the different forest divisions of Odisha after gathering the information regarding the edibility of these mushrooms. They were brought in the laboratory and surface sterilization was done by using 0.1% HgCl<sub>2</sub> solution, subsequently washing was done with the sterilized distilled water and tissue was transferred to the malt extract agar medium aseptically and incubated at 28 °C to get mushroom mycelium. Subsequent inoculations were done in order to get pure culture.

### Amylase activity

Starch agar media was used in order to screen the starch hydrolysis activity, inoculation of the respective mushroom mycelium was done and plates were incubated at 28°C. After the appreciable amount of the growth of mycelium, 1% iodine solution was added to the plates. Clear zone was observed for the organisms showing positive results.

### Cellulase activity

Sodium salt of carboxymethylcellulose (0.5%) was used as the substrate for this test. After the mycelial colonization, the plates were flooded with 0.2% Congo red solution, after the incubation period of 15 minutes plates were washed with 1M NaCl solution and clear zone was observed for the cellulase producing species.

### Lipase activity

Spirit blue agar media with tween- 20 was used for the screening of lipase activity. After the requisite amount of growth

of mushroom mycelium a clear precipitate was observed for the lipase producers.

### Protease activity

Gelatin agar media was used for the assessment of extracellular protease activity. After the required amount of the mycelial growth, plates were flooded with the reagent containing 15% HgCl<sub>2</sub> and 20% HCl. A visible zone was observed for the protease producing organisms.

### Xylanase activity

Medium containing xylan was used for the screening of Xylanase activity in mushroom species. After the appreciable growth of mushroom mycelia in the plate it was flooded with 0.1% Congo red, incubated for 30 minutes and washed with 1M NaCl solution. Plates were observed for the formation of clear zone for the positive organism.

### L-Asparaginase activity

For testing L- Asparaginase activity, medium containing 1% L- asparagine was used where it served as substrate, after the mycelial growth in the plate it was flooded with Nessler's reagent. Plates showing pink coloration after the addition were recorded as extracellular L- asparaginase producer.

### Organic acid production

Modified Sperber's medium was used for the screening of the mushroom mycelium for the production of organic acid where bromocresol green was used as a indicator, plates after the inoculation of the respective mushroom mycelium were incubated at 28 °C in triplicates, the acid production in the medium was determined by the yellow zone formed around the colonies.

### IAA production

The mushroom mycelium were inoculated in triplicates in a specific medium i.e JNF medium and pH 5.5 was maintained, the plates were incubated at 28 °C for 10 days. After observing proper growth in plates Salkowski' S reagent was added, pink

coloration in the medium confirmed the presence of IAA production Shrivastava et al. [34].

### Results and Discussion

Amylase is the widely distributed fungal enzyme used for the hydrolysis of starch, reports suggests that these enzymes are used for the fruiting body formation mainly in the ectomycorrhizal mushrooms Terashita et al.[35], Hur et al. [36], Hur et al. [37]. In the present studies only *M. procera* and *R. brevipes* showed a very little amylase activity where as no activity was found in rest of the mushroom species. Reports from Sławińska and Kalbarczyk [38] shows that cellulase activity in some *Pleurotus* species is more than amylase activity hence similar findings was recorded in the present studies in case of *R. brevipes*, *R. nigricans*, *R. lepida* and *C. indica*. A good cellulase activity was found in the *R. nigricans* while moderate activity was recorded in *R. brevipes*.

Fungal lipases are produced extracellularly and widely used in the food industry (Sharma et al., 2001). However, very less findings regarding lipolytic activity from the edible fungi has been reported such as *Agaricus bisporus* Wang et al. [39], *Lentinus edodes* Zhu et al. [40] and *Antrodia cinnamomea* Lin et al. [41] and Shu et al. [42] in the present studies lipolytic activity was seen in *R. brevipes* and *L. tuberregium* whereas no activity was found rest of the species. Protease is served as an industrially as well as pharmaceutically important enzyme which is distributed among the various sources Rao et al. [43] Choi and Shin [44], Choi and Sa [45] some mushroom species also produce protease enzymes Lee et al. [46], Park et al. [47], Kim et al. [48]. A very weak proteolytic activity was observed in *R. brevipes* whereas no such activity was seen in other studied mushroom mycelia.

In general it is considered that most of the microbial L-asparaginase is intracellular in nature except some, which is secreted outside the cell Savitri et al. [49], Narayana et al. [50]. However no records are available regarding some species which is presently studied such as *R. brevipes*, *R. nigricans* and *R. lepida* regarding the exogenous production of L-asparaginase. Present findings suggest that maximum L-asparaginase activity was recorded in *R. nigricans* and *R. brevipes* where as moderate production of the enzyme was recorded in *M. procera* and *R. lepida*.

Many researches demonstrate that IAA can be synthesized by utilizing tryptophan by plants and bacteria. Only few reports are there regarding phytohormonic potentialities of mushrooms, some reports from Tsivileva et al. [51] and Bose et al. [52] suggests that even mushrooms are also capable of synthesizing IAA if tryptophan is used as a substrate. In the present work good activity was shown by the *L. tuberregium*, moderate activity by *R. nigricans* and *C. indica* where as poor activity was recorded in *R. lepida* and *R. brevipes*.

Phosphate solubilization by some ectomycorrhizal mushrooms are important aspect to be studied, reports suggest that ectomycorrhizal mushrooms solubilises insoluble phosphate and helps plant for the P intake Arumanayagam and Arunmani

[53]. *R. nigricans* showed the best phosphate solubilization while in all the mushroom species studied no activity was found. Wild edible mushrooms are reported for the organic acid production Valentano et al. [54]. In the present screening, extracellular production of organic acid was seen only in *R. nigricans* where other species showed no such activity [55].

The availability of enzymes from mushroom species also remains a best viable option which needs to be further explored. Production of enzymes by the agro waste can used for production of the enzymes by the mushroom mycelium which may be the alternative path for the reuse of agro wastes, since in many cases agro wastes serves as a substrates for the mushroom cultivation. Presently screened enzymes can meet the industrial needs if the optimization of these enzymes will be done by amendment of nutritional and other factors. Preliminary screening of these mushroom species can provide a base work for the researchers to explore upto the purification and elucidation level.

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