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

Ashutosh Rajoriya and Nibha Gupta*
Department of Plant Pathology and Microbiology, Regional Plant Resource Centre, India

Submission: October 04, 2016; Published: December 14, 2016

*Corresponding author: Nibha Gupta, Department of Plant Pathology and Microbiology, Regional Plant Resource Centre, Bhubaneswar-751015, Odisha, India, Tel: 06742557925, Email: nguc2003@yahoo.co.in

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 potentials; 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 suggest Proteolytic activity Nakamura et al. [18], Terashita et al. [19], Nonaka et al. [20], Healy et al. [21], Cellulytic activity Kumar et al. [22], Madan and Bisara [23], amyrase 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     | R. lepida     | -       | -        | -      | +        | +         | ++             | -                      | +   | -                    |
| 2     | R. nigricans  | -       | -        | -      | -        | +++       | +++            | -                      | ++  | ++                   |
| 3     | R. brevipes   | +       | +        | +++    | -        | ++        | ++             | -                      | +   | -                    |
| 5     | V. volvacea   | -       | -        | -      | -        | -         | -              | -                      | -   | -                    |
| 6     | L. tuberregium| -       | -        | +++    | -        | -         | -              | -                      | +++ | -                    |
| 7     | C. indica     | -       | -        | -      | +        | +         | -              | ++                     | -   | -                    |

Materials and Methods

Collection and identification

Mushrooms species of *R. lepida*, *R. brevipes*, *R. nigricans*, *M. procera*, *V. volvacea*, *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% HgCl₂ 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% HgCl₂ 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 suggest 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 whereas 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 potencialities 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 solubilizes insoluble phosphate and helps plant for the P intake Arumanayagam and Arummani [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.

Acknowledgements

The financial assistance obtained from Ministry of Environment and Forests, Govt. of India (Project no. 22-24/2010 CS I) is gratefully acknowledged by the authors.

References

1. Abbas CA, Groves S, Gander JE (1999) Isolation, purification, and properties of Penicillium chartarose alkaline protease. J Bacteriol 171(10):5630-5637.
2. Beckle B, Galunsky B, Muller R (1995) Characterization of a keratinolytic serine protease from Streptomyces parvus DSM 40530. Appl Environ Microbiol 61(10): 3705-3710.
3. Friedrich J, Gradisar H, Mandin D, Chaunant JP (1999) Screening fungi for synthesis of keratinolytic enzymes. Lett Appl Microbiol 28(2):127-130.
4. Allpress JD, Mountain G, Gowland PC (2002) Production, purification and characterization of an extracellular keratinase from lysobacter NCIMB 9497. Lett Appl Microbiol 34(5):337-342.
5. Rao MB, Aparna M, Tankasale M, Ghatge S, Deshpande W (1998) Molecular and biotechnological aspects of microbial proteases. Microbiol Mol Biol Rev 62(3):597-635.
6. Kumar GG, Takagi H (1999) Microbial alkaline proteases: From a bio-industrial viewpoint. Biotechnol Adv 17(7):561-594.
7. Kirk O, Borchert TV, Fuglsang CC (2002) Industrial enzymes applications. Curr Opin Biotechnol 13(4):473-481.
8. Nehra KS, Singh A, Sharma J, Kumar R, Dhillon S (2004) Production and characterization of an alkaline protease from Aspergillus species and its compatibility with commercial detergents. Asian J Microbiol. Biotechnol Environ Sci 6(4):67-72.
9. Amozejegera MA, Fatemia AZ, Karbalaei-Heidari HR, Razavic MR (2007) Production of an extracellular alkaline metalloprotease from a newly isolated, moderately halophile, Salinibrio sp. strain AF-2004. Microbiol Res 162(4):369-377.
10. Forgyatt WM, Kelly CT (1983) In: Microbial enzymes and biotechnology. Environmental and Applied Science Publishers, pp. 131-182.
11. Singh A (1999) Engineering enzyme properties. Indian Journal of Microbiology 39(2):65-77.
12. Beg QK, Sahai V, Gupta R (2003) Statistical media optimization and alkaline protease production from Bacillus mojavensis in a bioreactor. Process Biochemistry 39(2): 203-209.

13. Eliajah P, Adinarayana K, Rajyalakshmi P, Srihvanalu B (2003) Optimization of process parameters for alkaline protease production under solid state fermentation by alkalophilic Bacillus sp. Asian J Microbiol Biotechnol Environ Sci 5: 49-54.

14. Nascimento WCA, Martins MLI (2004) Production and properties of an extracellular protease from thermophilic Bacillus sp. Braz J Microbiol 35(1-2): 91-96.

15. Gouda MK (2006) Optimization and purification of alkaline proteases produced by Marine Bacillus sp. MIG newly isolated from eastern harbor of Alexandria. Polish J Microbiol 55(2): 119-126.

16. Ho I, Zak B (1979) Acid phosphatase activity of ectomycorrhizal fungi. Can J Botany 57(11): 1203-1205.

17. Elizair F, Tabatabai MA (1988) Glucosidases and galactosidases in soils. Soil Biology and Biochemistry 20(5): 601-606.

18. Nakamura M, Iketani A, Shioi Y (2011) A survey of proteases in edible mushrooms with synthetic peptides as substrates. Mycoscience 52(4): 234-241.

19. Terashita T, Kono M (1989) Purification and some properties of carboxyl proteinase from Tricholoma matsutake. Trans Mycol Soc Japan 28(3): 245-256.

20. Nonaka T, Ishikawa H, Tsumuraya Y, Hashimoto Y, Dohmae N, et al. (1995) Characterization of a heat-stable l-seryl-specific metalloendopeptidase from the fruiting bodies of a basidiomycete, Grifola frondosa. J Biochem 118(5): 1041-1042.

21. Healy V, O‘Connell J, McCarty TV, Doonan S (1999) The l-seryl-specific protease from Armillaria mellea is a member of a novel class of metalloendopeptidases located in Basidiomycetes. Biochim Biophys Acta Commun 262(1): 60-63.

22. Kumaran S, Sastry CA, Vikneswary S (1997) Laccase, cellulase and xylanase activities during growth of Pleurotus sajor-caju on saucha-pattam. World Journal of Microbiology and Biotechnology, 13(1): 43-49.

23. Madan M, Bisaria R (1983) Cellulytic enzymes from an edible mushroom, Pleurotus sajor-caju. Biotechnology Letters 5(9): 601-604.

24. Jonathan SG, Adeoye OR (2011) Evaluation of ten wild Nigerian mushrooms for amylase and cellulase activities. Mycobiology 39(2): 103-108.

25. Ghosh AK, Banerjee PC, Sengupta S (1980) Purification and properties of xylan hydrolase from mushroom Termitomyces cypeatus. Biochim Biophys Acta 612(1): 143-152.

26. Lee JW, Gwak KS, Kim SL, Kim M, Choi DH (2007) Characterization of xylanase from Lentinus edodes M290 cultured on waste mushroom logs. J Microbiol Biotechnol 17(1): 1811-1817.

27. Eisele N, Linke D, Bitzer K, Na’amnieh S, Nimtz M, et al. (2011) The first characterized asparaginase from a basidiomycete, Flammulina velutipes. Bioresour Technol 102(3): 3316-3321.

28. Mishra RC (2011) Estimation of Enzymatic Activities of Different Species of Mushrooms. MSc thesis, National Institute of Technology, Rourkela, India.

29. Shu CH, Xu CJ, Lin GC (2006) Purification and partial characterization of a lipase from Antrodoa cinnamomea. Process Biochemistry 41: 734-738.

30. Bose A, Shah D, Keharia H (2013) Production of indole-3-acetic-acid (IAA) by the white rot fungus Pleurotus ostreatus under submerged condition of Jatropha seedcake. Mycology 4(2): 103-111.

31. Lapeyrre E, Ranger J, Vairelles D (1991) Phosphate-solubilizing activity of ectomycorrhizal fungi in vitro. Canadian Journal of Botany 69(2): 342-346.

32. Levyval C, Berthelin J (1989) Interactions between Laccaria laccata, Agrobacterium radiobacter and beech roots: Influence on P, K, Mg, and Fe mobilization from minerals and plant growth. Plant and Soil 117(1): 103-110.

33. Largent DL (1981) How to identify mushrooms to genus I: Macroscopic features.

34. Shrivastava UP, Kumar A (2011) A simple and rapid plate assay for the screening of indole 3-acetic acid (IAA) producing microorganisms. International Journal of Applied Biology and Pharmaceutical Technology 2(1): 120-123.

35. Terashita T, Kono M (1989) Purification and some properties of carboxyl proteinase from Tricholoma matsutake. Trans Mycol Soc Japan 28(3): 245-256.

36. Hur TC, Park H, Chang JH, Joo SH (1998) Changes in soil physicochemical properties and dehydrogenase activity by the formation of fairy ring of Tricholoma matsutake. Journal of Korean Forestry Society 87(2): 270-275.

37. Hur TC, Ka KH, Joo SH, Terashita T (2001) Characteristics of the Amylase and its Related Enzymes Produced by Ectomycorrhizal Fungus Tricholoma matsutake. Mycobiology 29(4): 183-189.

38. Slaievića A, Kalbarczyk J (2011) Evaluation of enzymatic activity of Pleurotus ostreatus regarding stages of mycelium development. Acta Pol 10(2): 195-202.

39. Wang ZS, Liao JH, Li FG, Wang HC (1991) Studies on the genetic basis of esterase isozyme loci Est A, B and C in Agaricus bisporus. Mushroom Sci 13(1): 3-9.

40. Zhu J, Huang Y, Jiang P (2000) The isozyme pattern of esterase and fuzzy clustering analysis of Lentinus edodes strains. Pujian J Agric Sci 15: 46-50.

41. Lin ES, Ko HC (2005) Glucose stimulates production of the alkaline-thermo-stable lipase of the edible Basidiomycete Antrodoa cinnamomea. Enzyme and Microbial Technology 37(2): 261-265.

42. Narayana R, Narayana R, Vijayalakshmi M (2008) L-asparaginase production by Streptomyces abhido-flavus. Indian Journal of Microbiology 48(3): 331-336.

43. Rao MB, Aparna M, Tanksale M, Ghatge S, Deshpande VV (1998) Molecular and biotechnological aspects of microbial proteases. Microb Mol Biol Rev 62(3): 597-635.

44. Choi HS, Shin HH (1998) Purification and characterization of cysteine protease from Pleurotus ostreatus. Bioscience Biotechnology Biochemistry 62(7): 1416-1418.

45. Choi HS, Sa YS (2000) Bifibrinolytic and antithrombotic protease from Ganoderma lucidum. Mycologia 92(3): 545-552.

46. Lee SY, Kim JS, Kim JE, Sapkota K, Shen MH, et al. Purification and characterisation of fibrinolytic enzyme from cultured mycelia of Armillaria mellea. Protein Expr Purif 43(1): 10-17.

47. Park SE, Li MH, Kim JS, Sapkota K, Kim J et al. (2007) Purification and characterization of a fibrinolytic protease from a culture supernatant of Flammulina velutipes mycelia. Bioscience, Biotechnology, and Biochemistry 71(9): 2214-2222.

48. Kim JS, Kim JE, Choi BS, Park SE, Sapkota K, et al. (2008) Purification and characterization of fibrinolytic metalloprotease from Perenniporia fraxinea-mycelium. Mycological Research 112(8): 990-998.

49. Savitri A, Stana N, Azmi W (2003) Microbial L-asparaginase. A potent antitumour enzyme. Indian Journal of Biotechnology 2: 184-194.
50. Narayana KJP, Kumar KG, Vijayalakshmi M (2008) L-asparaginase production by Streptomyces albido-flavus. Indian Journal of Microbiology 48(3): 331-336.

51. Tsivileva OM, Loshchinina EA, Nikitina VE (2013) The Extracellular Indolic Compounds of Lentinus edodes. Environmental Biotechnology - New Approaches and Prospective Applications pp: 217-234.

52. Bose A, Shah D, Keharia H (2013) Production of indole-3-acetic-acid (IAA) by the white rot fungus Pleurotus ostreatus under submerged condition of jatropha seedcake. Mycology 4(2): 103-111.

53. Arumanayagam S, Arummani M (2014) Rock Phosphate solubilization by the ectomycorrhizal fungus Laccaria fraterna and its associated helper bacterial strains. African Journal of Biotechnology 13(25): 2524-2530.

54. Valentao P, Lopes G, Valente M, Barbosa P, Andrade P et al. (2005) Quantitation of Nine Organic Acids in Wild Mushrooms. J Agric Food Chem 53(9): 3626-3630.

55. Kumar CG, Takagi H (1999) Microbial alkaline proteases: from a bioindustrial viewpoint. Biotechnol Adv 17(7): 561-594.

Your next submission with JuniperPublishers will reach you the below assets

• Quality Editorial service
• Swift Peer Review
• Reprints availability
• E-prints Service
• Manuscript Podcast for convenient understanding
• Global attainment for your research
• Manuscript accessibility in different formats (Pdf, E-pub, Full Text, audio)
• Unceasing customer service

Track the below URL for one-step submission
http://juniperpublishers.com/online-submission.php