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Characterization of Mushroom Strains on the Basis of Physiological Conditions and Enzymatic Activity

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A B S T R A C T

A study on 18 strains of mushrooms belonging to 5 genera namely *Agaricus*, *Calocybe*, *Lentinula*, *Pleurotus*, *Tricholoma*was done for biochemical and physiological studies. These strains were obtained from Directorate of Mushroom Research, Solan and Mushroom Lab, RPCAU, Pusa. Radial growth measurement of mycelium for optimum growth conditions for all the strains were recorded with respect to different media, temperature and pH. The biochemical screening of enzymatic activities for all the strains was also done for lipase, amylase, laccase and oxidase.

Introduction

Just the way a seed needs proper environment to grow, microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce (Ravimannan *et al.,* 2014). Growth in pure mycelium culture in a solid medium is the first step of cultivation of edible mushrooms. Mycelial growth into a solid substrate is affected by various factors, including temperature, pH, nutrient ingredients and environmental factors (Imtiaj *et al.,* 2008), the time required for the growth of mycelium on a solid medium is relatively short, and thus an accurate and quick assessment can be facilitated. Therefore, the quality and quantity of a mushroom strain mycelium in a solid medium is one of the most important matrices to determine further nutritional requirements for the production of fruiting bodies of the mushroom. (Masoumi, 2015) and mycelial growth rate could be used as a criterion to select for fast-growing isolates, as it is

assumed that mushroom fast-growing strains colonize compost or casing layer much faster and achieve high production yields compared to the slower strains (Guadarrama-Mendoza *et al.*, 2014).

During the growth of mushroom mycelia and the development to mature fruitbodies (or sporophores), biochemical changes are known to occur, as a result of which enzymes are secreted extracellularly to degrade the insoluble materials in the substrates into simple and soluble molecules which are subsequently utilized by intracellular enzymes within the mushroom. Consequently, enzymes play significant role in mushroom development; in addition, they also affect the food nutrient, flavour and shelf life of these fungi (Baardseth, 1979; Paranjpe and Chen, 1979; Wang, 1989; Zadrazil *et al.*, 2004; Kuforiji and Fasidi, 2008; Kapoor *et al.*, 2009). It is considered that, with few exceptions, ectomycorrhizal Basidiomycota do not have enzymes that can degrade cellulose and lignin (Hutchison 1990; Tedersoo *et al.*, 2010), although in this respect, final conclusions have not yet been drawn. However, some ectomycorrhizal fungi, such as *Tricholoma aurantium, Amanita muscaria, Rhizopogon luteolus, Rhizopogon roseolus* and *Cenococcum geophylum* (Trojanowski *et al.*, 1984), are able to degrade lignin and cellulose, so they act as successful competitors to saprotrophs (Koide *et al.*, 2008; Dames *et al.*, 1999). Due to this ability, some ectomycorrhizal fungi are able to survive after lengthy dry periods or harmful disturbances in the ecosystem (Dames *et al.*, 1999).

Materials and Methods

Fungal material

The morphological and cultural characteristics of vegetative mycelium of 18 mushroom strains were investigated for morphological, cultural and biochemical characterization. The fungal materials were maintained by sub-culturing throughout the study (Table.1.)

Media

Six different media were used for the study viz. Potato Dextrose Agar (PDA), Wheat extract Agar (WEA)and Malt Extract Agar (MEA), Starch Agar Medium (For Amylase Test), Lipase Test Medium, Laccase Test Medium.

Oxidase discs

Oxidase test was performed using oxidase discs from Micromaster Laboratories Pvt. Ltd. The wet discs would change color within seconds of coming in contact with the culture as a positive test for oxidase.

Results and Discussion

Media

The radial growth(mm) was calculated on 3 different media viz. Potato Dextrose Agar, Wheat extract Agar and Malt Extract Agar after 7 days of inoculation in 3 replicates. Highest radial growth was observed in PL-17-02 in WEA which was at par with PL-17-01 in the same medium. Among all the strains PL-17-02 showed the best radial growth and WEA was statistically the best medium. It was observed that *Tricholoma* strains achieved the best radial growth on PDA (50 mm by CiP-18 and 39 mm by CiP-19) followed by WEA and least on MEA. *Agaricus* strains grew best on MEA (AB-14-01 and AB-16-02 attained 19 mm) followed by PDA. *Lentinula* strains grew best on PDA ranging from 34 mm in LE-1503 to 40 mm in LE-15-01 and LE-15-04 followed by WEA (Figure 1).

Temperature

The radial growth (mm) was calculated after 7 days of inoculation on 3 replicates of PDA medium for 6 different temperature ranging from 15°C to 40°C with a difference of 5 degrees. Highest radial growth was observed in PF at 15°C which was at par with PD at the same temperature and PF and PL-17-03 at 20°C. Among all the strains PF and PL-17-03 showed the best radial growth and 15°C was statistically the best temperature (Figure 2).

It was observed that *Tricholoma* strains achieved the best radial growth at 25°C (50 mm by CiP-18 and 39 mm by CiP-19) and least at 15°C. It showed more than average growth even at 40°C. *Agaricus* strains on an average showed best results at 25°C (AB-14-01 and AB-16-02 attained 19 mm and 18 mm respectively) but these strains individually had different best temperatures i.e. 15°C for AB-16-02 and 20°C for AB-14-01. *Lentinula* strains grew best at 15°C with their radial growth ranging from 64 mm in LE-15-03 to 57 mm in LE-15-01. The growth declined with decrease in temperature and no growth was observed at 40°C. *Calocybe* strains attained maximum growth at 25°C with highest growth of 45 mm in CI-17-04 followed by 43 mm by CI-17-06 and 40 mm by CI-17-02. Individually 30°C was found best for CI-17-02 and C-17-04. *Pleurotus* strains grew at all the six temperatures with an average growth more than all the other strains but statistically grew best at 15°C showing a growth range from 83 mm in PL-17-01 to 90 mm in PD and PF, however the best temperature for strain PL-17-03 was found to be 20°C with 90mm of radial growth.

pH

Highest radial growth was observed in PF at pH 9. Among all the strains PL-17-03 showed the best radial growth and pH 7was statistically the best pH. The *Tricholoma* strains achieved the best radial growth at pH 9 (50 mm by CiP-18 and 49 mm by CiP-19) followed by pH 7.It showed more than average growth even at pH 5. *Agaricus* strains attained best results at pH 9 (AB-14-01 and AB-16-02 attained 19 mm and 23 mm respectively).They showed more than average growth even at pH 5. *Lentinula* strains were found to be pH sensitive and did not grow at pH 5 and pH 9, attained growth only at pH 7 ranging from 40 mm in LE-1501 and LE-15-04 to 34 mm in LE-15-03.*Calocybe* strains attained maximum growth at pH 7 with highest growth of 45 mm in CI-17-04 followed by 43 mm by CI-17-06 and 40 mm by CI-17-02 but individually pH 9 was best for CI-17-02 as it attained growth of 42 mm. *Pleurotus* strains on an average grew best at pH 9 showing a range of growth from 85 mm in PF to 70 mm in PSC, however the best pH for strain PD was found to be pH 7 with 60 mm of radial growth(Figure. 3).

Biochemical test

Enzymatic screening of mushroom strains was qualitatively done using differential agar plates and oxidation discs. A total of four tests pertaining to different activities was done on mushroom mycelium. The measurement of reaction by the strains was denoted by '–' for absence of the enzymatic activity, '+' for presence, '++'for strongly present, '+++' very strongly present and '++++' for excellent activity. The results of the experiments have been represented in Table 1.The positive test for each enzyme were as follows; for amylase the zone of clearance against iodine was checked; fragmentation of agar plate was observed for lipase test; the change in color of oxidase disc for oxidase test and pigmentation for the laccase test (Figure 4).

All the strains of *Pleurotus* and *Calocybe* showed positive test for amylase activity whereas all the strains of *Tricholoma*,

Lentinula and *Agaricus* showed negative results with AB-14-01 as an exception. Lipase was the only enzyme whose activity was found to be present in all the strains as it showed positive results for all the strains. Most of the strains were oxidase positive except for LE-15-03 and LE-1504. All the strains showed positive Laccase test except for three strain viz. CiP-18, CI-17-04, PL-17-01. AB-14-01 was found to be the strain with the best enzymatic activity followed by PF-1 and CI-17-06.

Table.1 List and source of strains used in the study

Sr.No.	Strain	Species	Source
1	CiP18	*Tricholoma giganteum*	R.P.C.A.U , Pusa
2	CiP19	*Tricholoma giganteum*	R.P.C.A.U , Pusa
3	AB-14-01	*Agaricus bisporus*	R.P.C.A.U , Pusa
4	AB-16-02	*Agaricus bisporus*	R.P.C.A.U , Pusa
5	LE-15-01	*Lentinula edodes*	D.M.R , Solan
6	LE-15-02	*Lentinula edodes*	D.M.R , Solan
7	LE-15-03	*Lentinula edodes*	D.M.R , Solan
8	LE-15-04	*Lentinula edodes*	D.M.R , Solan
9	CI-17-02	*Calocybe indica*	D.M.R , Solan
10	CI-17-04	*Calocybe indica*	D.M.R , Solan
11	CI-17-06	*Calocybe indica*	D.M.R , Solan
12	PL-17-01	*Pleurotus florida*	D.M.R , Solan
13	PL-17-02	*Pleurotus florida*	D.M.R , Solan
14	PL-17-03	*Pleurotussajor-caju*	D.M.R , Solan
15	PF	*Pleurotus florida*	R.P.C.A.U , Pusa
16	PF-1	*Pleurotus florida*	R.P.C.A.U , Pusa
17	PD	*Pleurotus djamor*	R.P.C.A.U , Pusa
18	PSC	*Pleurotus sajor- caju*	R.P.C.A.U , Pusa

Figure.1 Radial growth (mm) of mushroom strains at different media

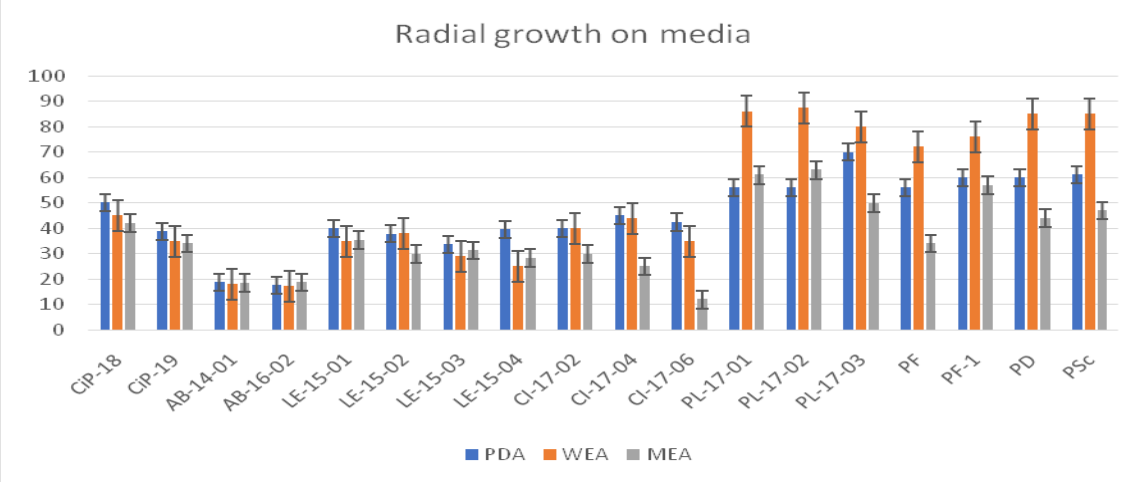


Figure.2 Radial growth (mm) of mushroom strains at different temperature

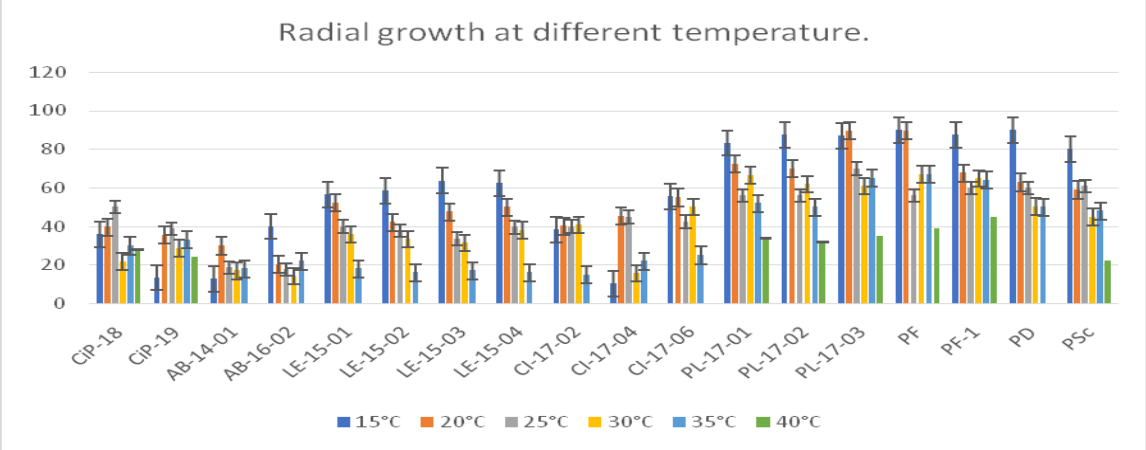


Figure.3 Radial growth (mm) of mushroom strains at different pH

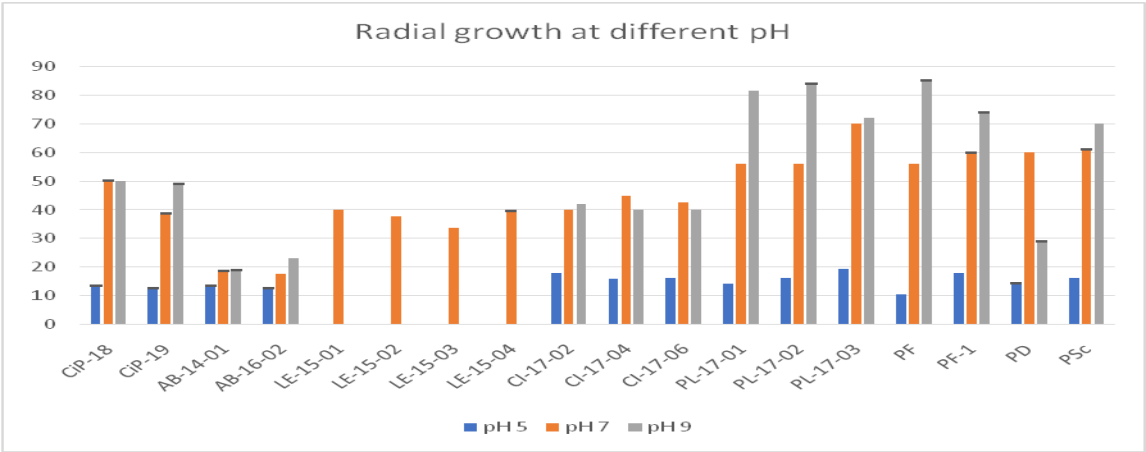
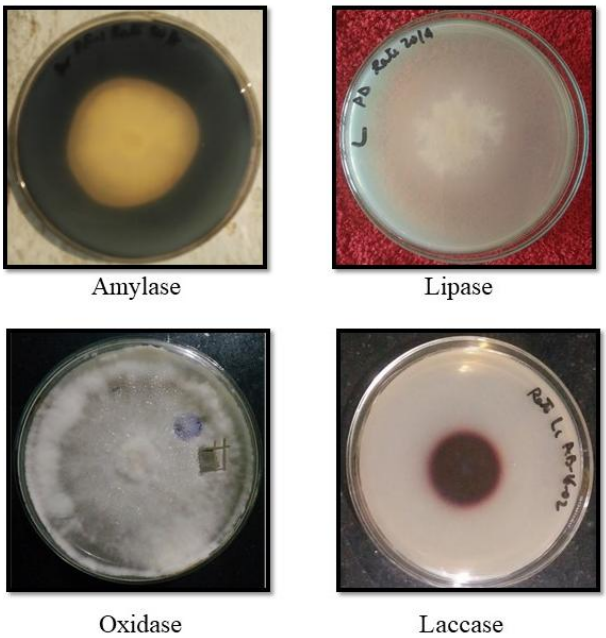


Figure.4 Biochemical tests



Media

The difference of mycelial growth on different agar media may be due to availability of different carbon sources and other required nutrients (Sardar, 2017). Growth of *Tricholoma* on different media was not reported earlier. The results for *Agaricus* deviated from Furlan *et al.,* (1997), who investigated higher on WDA (wheat/dextrose/agar) medium than on PDA (potato/dextrose/agar) or MPA (malt/ Soya peptone/agar) media in all strains. *Lentinula* strains results are comparable to that of Arif *et al.,* (2015) and Gbolagade *et al.,* (2006) findings, who observed that potato dextrose agar is best for the culture establishment of shiitake mushroom. The present findings for *Calocybe* are almost similar to the results obtained by Singh *et al.,* (2009), they reported that *C.indica* grew well on all the tested media but maximum growth was attained on WEA followed by PDA. *Pleurotus* strains results show a little deviation with the findings of Gibriell *et al.,* (1996); Hussain and Hussain, (2004) who reported that *Pleurotus* spp. showed fastest growth of mycelium on potato dextrose agar among different media used. The effect of carbon sources on mycelium growth depends on the fungal strains, its comparison of the growth of strains in solid media shows the presence of significant differences and erratic growth patterns between them (Akata *et al.,* 2012).

Temperature

The results for *Tricholoma*were similar to previous study which stated that *T. imbricatum, T. batchii* and *L. deliciosus,* were more thermophilic and grew faster at 25°C (Lazarević, 2016). *Agaricus* strains had results similar to Yadav (2014) which reported temperature of 25º C as more favorable to the mycelium growth of all A. bisporus strains. *Lentinula* results deviated from the present study as *Lentinula edodes Le-17-04* grew best 24°C (Kumar, 2019) as presently standardized cultural conditions for shiitake mushroom is unavailable (Kumar, 2019).The results for *Calocybe* strains are in accordance with the findings of Varshney (2007) and Kerketta (2017) who reported that of 25-35°C is the optimum temperature requirement for mycelial growth in *C. indica* while Shukla *et al.,* (2013) reported that 30°C was the best temperature for *C.indica*. Calocybe strain findings were a little similar to Wei *et al.,* (2002) who reported a temperature range of 20-31ºC for the hyphal growth of *P. flabellatus* and concluded that a temperature of 25ºC is the optimum. Similarly, Zharare *et al.,* (2010) found that *P.sajor-caju* can tolerate high 35ºC temperature.

pH

pH is important to study for the growth of mycelium pH and temperature effects the growth of mushroom through affecting the enzyme activity in the cell (Sopit, 2006);

The results for *Tricholoma* were almost similar to Lazarević (2016) stated that examined isolates were tolerant to different pH values, but all of them grew more rapidly at pH 5.8, consistent with the pH values measured at the sampling locality , thus the strains may have a change in pH with the change in locality. *Agaricus* strain results mirrored with Yadav (2014) as mycelial growth on 9 pH was fastest for all *A.bisporus* strains. *Lentinula* strains had similar findings to Arif *et al.,* (2015) which showed best mycelial growth of shiitake mushroom on 6 pH but statistically it was at par with 7 pH and almost all mushrooms perform and grow best at neutral pH (Khan *et al.,* 2013).The results or *Calocybe*have been very similar with the findings of Singh *et al.,* (2015), Shukla and Jaitly (2013) and Varshney (2007)

maximum mycelial growth was at pH7.5 followed by pH8. Some *Pleurotus* spp. are characterized for wider growth adaptability scale for pH i.e. 5-8 as reported by Yadav (2001). The mycelial growth of *P. ostreatus* was recorded best at pH 7.0 by Bugarski *et al.,* (2000). Singh (2017) reported that 7.5 was the best pH for the growth of *P. djamor*.

Biochemical Studies

Positive results for amylase can be explained as the examined isolates were capable of growing on polysaccharides, such as dextrin and starch, indicating the presence of enzymes that are able to carry out their decomposition to glucose (Lazarević, 2016). Lipase was the most effective enzyme for all the strains, it has been so consistent that Lipase was isolated from oyster mushroom (Wijayati *et al.,* 2017) also. Most of the strains were oxidase positive except for LE-15-03 and LE-15-04. However, it was difficult to track previous finding with respect to oxidase test in all the species. All the strains showed positive Laccase test except for three strain viz. CiP-18, CI-17-04, PL-17-01. It is a white rot basidiomycete which belongs to the subclass of ligninolytic microorganisms that produce laccases, manganese peroxidases, amylase, cellulase, pectinase and protease (Rashad and Abdou, 2001; Palmieri *et al.*, 2001; Abdou, 2003; Fan *et al.*, 2008 and Rashad *et al.*, 2009) This supports the present finding as all the strains under study belong to Basidiomycete. In previous studies also some strains showed negative test for laccase as mentioned by Kalmis (2008).

In conclusion, the optimum media, temperature and pH for the growth of mushroom strains used in the study was dependent on the strain of mushroom. The optimum growth conditions for *Tricholoma* strains were PDA medium, 25°C of temperature and pH 9 while for *Agaricus* MEA medium, 25°C of temperature and pH 9 were more appropriate. PDA medium, 15°C temperature and pH 7 was suitable for *Lentinula* strains and WEA medium, 15°C temperature and pH 9 for *Pleurotus* strains. *Calocybe* strains showed best growth on both PDA and WEA, at 25°C and pH 7. Overall the best media for all the strains was WEA, best temperature 15°C and best pH was 7. Based on the present study *Lentinula* strains were identified to be pH sensitive.

Most of the strains showed positive results for lipase, oxidase and laccase activity. All the strains of *Pleurotus* and *Calocybe* showed positive results for amylase activity however it was absent in the strains of *Tricholoma* and *Lenitnula*. The strain AB-14-01 of *Agaricus* was identified as the best strain for all the enzymes. As AB-14-01 showed maximum enzymatic activity, it can be used for bioremediation.

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