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Nutritional requirements for growth and ligninolytic enzymes production by *Chondrostereum purpureum*

I.B. PRASHER AND SHEHNAZ

Mycology and Plant Pathology Laboratory, Department of Botany, Panjab University, Chandigarh, 160014, India

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ABSTRACT

Chondrostereum purpureum (Pers. ex Fr.) Pouzar is a wood-inhabiting white-rot fungus frequently found in temperate regions. It is commonly employed against sprouting of broad-leaved trees. The objective of this study is to examine the growth and ligninolytic enzymes production by fungi with various carbon and nitrogen compounds. The results revealed that D(-) xylose and maltose supplemented media supported the optimum growth of Chondrostereum purpureum. MnP production was higher in medium supplemented with pectin and laccase with D (-) xylose. Amongst the inorganic and organic nitrogen compounds, maximum mycelial growth was noticed in medium augmented with ammonium chloride and L-cystine, respectively. LiP and MnP production was higher in media supplemented with ammonium acetate. Laccase production was higher in media supplemented with ammonium phosphate. In case of organic nitrogen compounds, LiP production was higher in medium supplemented with DL-methionine, MnP in media supplemented with L-ornithine HCl and laccase with DL-serine HCl.

Keywords: *Chondrostereum purpureum*, sprouting, ligninolytic enzymes

INTRODUCTION

Chondrostereum purpureum (Pers. ex Fr.) Pouzar is a lignicolous wood-inhabiting and wood decaying white-rot agaricomycete fungi found worldwide especially in boreal and temperate vegetation zones (Hamberg *et al.* 2014). It has been studied for its unique ecology and pathology (Roy *et al.* 2010). It is a facultative saprophyte as it has the broad spectral pathogenicity towards many hardwood tree species and is utilized as a biocontrol agent against prolific sprouting and root suckering of broad-leaved trees such as sugar maple, red maple, red alder, bigleaf maple, yellow birch, paper birch, beech, trembling aspen, bigtooth aspen, pin cherry and black cherry (Becker *et al.* 2005). Fungus affects the trees only through fresh open wounds. Then it grows through the xylem tissue of the host plant, leading to decay, cambial necrosis, sapwood staining and occasionally may cause death of the host (Wall, 1991). The infection by *C. purpureum* can further cause foliar discoloration or silvering because of which this fungus is also known as silver-leaf fungus (Becker *et al.* 2005). As *C. purpureum* is a white-rot fungus, so it is capable of breaking down the lignin. As in woody cell walls, lignin surrounds the cellulose which is the basic carbon and energy source for the fungus and basic reason for lignin degradation. Laccase is the oxidative enzyme, capable of this deterioration process besides lignin and manganese peroxidase (Hamberg *et al.* 2015). Ulcnik *et al.* (2012) examined the ability of four white-rot fungi including *Chondrostereum purpureum* to degrade the organochlorine insecticide lindane in liquid cultures. Reina *et al.* (2017) for the first time examined the detoxification of dry olive mill residue by using three agaricomycetes fungi such as *Chondrostereum purpureum, Cyclocybe aegerita* and *Mycetinis alliaceus* due to their lignocellulolytic enzymes secretion and the results reveals that *Chondrostereum purpureum* eliminated dry olive mill residue phytotoxicity. Since any biotechnological process is likely to be based on crude enzymes, so it is important to increase their activities in the culture supernatants by choosing the finest carbon and nitrogen sources (Gao *et al.,* 2008). Recent researches have revealed that culture conditions could influence fungal physiology and expression of the ligninolytic enzymes (Hou *et al.,* 2004). Therefore, the laboratory study was carried out to find the nutritional requirements of *Chondrostereum purpureum* for growth and ligninolytic enzymes production.

MATERIALS AND METHODS

Chondrostereum purpureum culture was obtained from IMTech., (MTCC 1018) Chandigarh (India) and cultured in Petri dish containing corn meal agar (CMA) (17.0 gram corn meal agar and chloramphenicol 0.1g in distilled water to make 1000ml) and incubated at 24°C. The culture was maintained in CMA media prior to use and stored at 4°C. Experiments were performed in 100ml Erlenmeyer flasks having 25ml media (autoclaved at 15 psi pressure for 15-20 minutes). The flasks were inoculated with mycelium of a 10- day old culture and three replicates were kept for each parameter. Mycelial dry weight was determined after each experiment by filtering through Whatman filter paper No.1. Mycelium was dried at 50°C in a hot air oven and electronic balance (Ohaus Pioneer) was used to determine the dry weight of mycelium. The final pH of the culture filtrate was also noted down for each replicate (pH Meter 813). Experiments were performed in Mycology and Plant Pathology laboratory of Botany Department, Panjab University, Chandigarh in year 2017. The carbon compounds employed for experiment were, D(+) glucose, maltose, sucrose, D(+) lactose, pectin, D(+) raffinose, L(-) sorbose, D(-) fructose, starch, and D(+) xylose. The experiment was carried out at optimized conditions (20 days of incubation at 24°C, pH 5.0). Control was taken without carbon source. The glucose of the glucose peptone medium (media supported the optimum growth) was substituted singly by each of the carbon compound so as to provide 0.333g/L of carbon - a substituent of glucose (10g/L) in the basal medium.

Effect of nitrogen compounds were studied by replacing the peptone with inorganic and organic nitrogen compounds (2.0g/L) for mycelial biomass production and ligninolytic enzymes production. The experiment was carried out under optimum conditions (at 24°C and pH 5.0 in D(+) xylose supplemented basal medium for 20 days of incubation) and control (without nitrogen) was run in parallel. Laccase (Coll *et al.,* 1993), MnP and LiP activity were (Atalla *et al.,* 2010) assayed using SHIMADZU UV spectrophotometer 1800. All the experiments were performed in triplicates and the mean of three replicate values for all data in the experiments obtained were tested in a one way ANOVA at P=0.05 using PASW Statistics 18 software and Tukey's test was used to evaluate differences between treatments.

RESULTS AND DISCUSSION

Impact of Carbon Compounds

Different fungi require different nutrients for its growth as all fungi are not able to utilize same substrate for their growth. Carbon is the most important constituent for the growth of fungi. It constitutes about half of total dry weight of fungi (38-50%) (Zhang and Elser, 2017). Different fungi utilize different carbon sources for their growth and development. The optimum growth of fungi was observed in media supplemented with D(+) xylose and maltose followed by D(-) fructose, starch, D(+) lactose, D(+) raffinose, D(+) glucose, L(-) sorbose, sucrose and pectin (Fig. 1). Juwon and Emmanuel (2012) also observed the highest growth of *Trichoderma viride* in maltose supplemented media and supplementation of culture medium with maltose also supported the optimum growth of *Pleurotus ostreatus* 108 (Mikiasvili *et al.,* 2006). The final pH of the culture filtrate was shifted towards alkalinity with all carbon compounds. Fungus didn't exhibit LiP activity with any of the carbon compound, whereas MnP production was observed in medium supplemented with pectin. Laccase activity was high in medium supplemented with D(+) xylose, sucrose, maltose, D(+) raffinose, D(+) glucose, D(+) lactose and D(-) fructose, whereas starch and pectin supplemented media exhibited less activity (Fig. 2). Two strains of *Pleurotus ostreatus* expressed the laccase activity with glucose supplemented media (Stajic *et al.,* 2006).

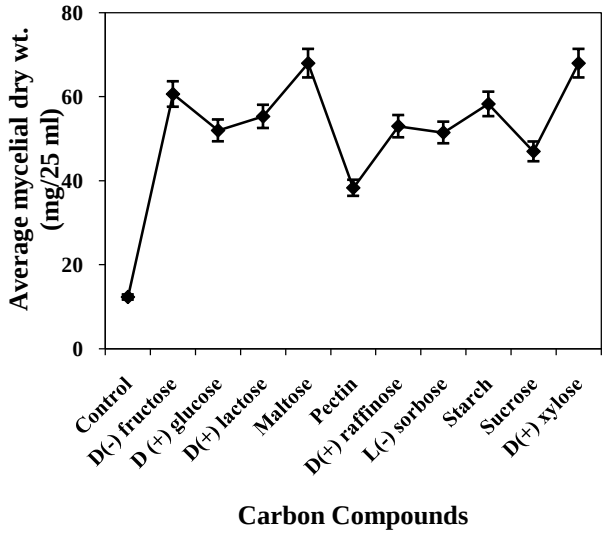

Fig.1. Growth rate (average mycelia dry wt. mg/25ml) with different carbon compounds

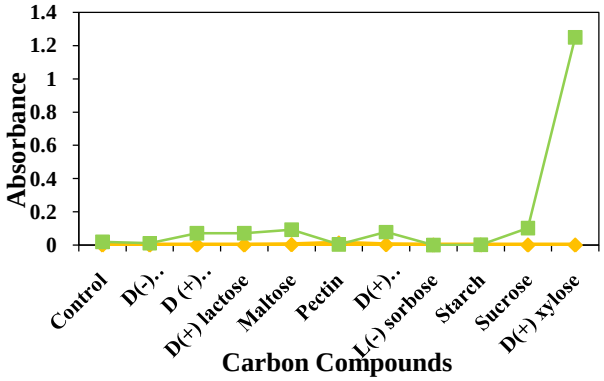

Fig.2. Ligninolytic enzyme production by *Chondrostereum purpureum* with different carbon compounds

Impact of Inorganic Nitrogen Compounds

Nitrogen plays a significant role in the physiology of fungi and thus gained a lot of attention, it constitute 38% of total dry mass. The optimum growth of fungi was observed in medium supplemented with ammonium chloride followed by ammonium phosphate and sodium nitrate. *Dictyoarthrinium synnematicum* expressed the highest growth with sodium nitrate supplemented media (Prasher and Chauhan, 2015). Among all the inorganic nitrogen, media supplemented with sodium nitrite didn't support the mycelium growth (Figure 3). Similar to our findings *Porostereum spadiceum* also showed the least growth with sodium nitrite supplemented media (Prasher and Manju, 2018). The pattern of biomass and enzymes production was different, fungi showed higher production of LiP and MnP in ammonium acetate supplemented media. Laccase production was predominant than LiP and MnP, in this experiment and was high in media supplemented with ammonium phosphate followed by ammonium sulphate (Fig. 4).

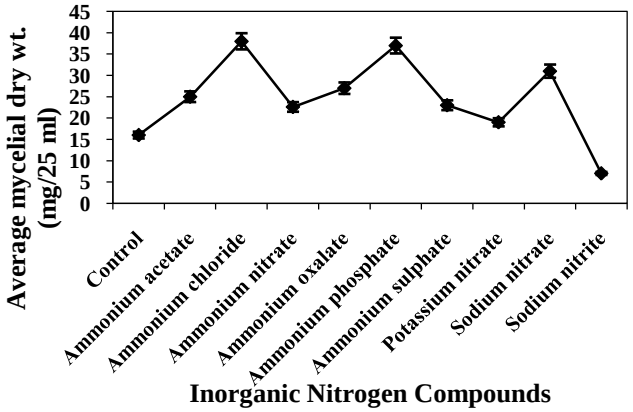

Fig.3. Average mycelial dry wt. mg/25ml of *Chondrostereum purpureum* with different inorganic nitrogen compounds

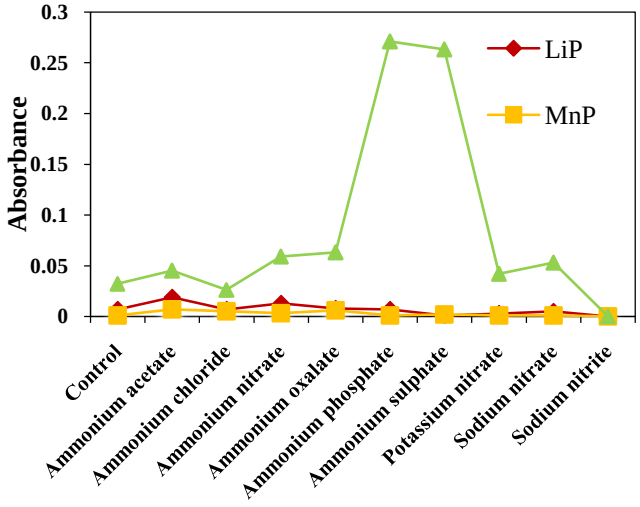

Fig.4. Ligninolytic enzymes production of *Chondrostereum purpureum* in relation to different inorganic nitrogen compounds

Impact of Organic Nitrogen Compounds

Fungus showed the optimum growth in medium supplemented with L-cystine followed by L-asparagine, Glycine, L-cysteine HCl and DL-aspartic acid (Fig. 5). During the study it was observed that organic nitrogen compounds affects the growth of fungi differently. L-tryptophan significantly supported the growth of *Psathyerella atroumbonata* (Jonathan and Fasidi, 2001), whereas Alanine supplemented media supported the growth of *Neolentinus kauffmanii* (Johnsy and Kaviyarasan, 2014). The study regarding production of ligninolytic enzymes showed the LiP production was high in medium supplemented with DL-methionine followed by DL-histidine and DL-tryptophan. MnP production was high in media supplemented with L-ornithine HCl followed by Glycine, DL-tryptophan, DL-methionine, L-arginine HCl and DL-serine HCl. Laccase activity was high in medium supplemented with DL-serine HCl and L-leucine and DL-methionine (Fig. 6). Study on effects of various amino acids on laccase production by *Cyathus bulleri* was carried out and has shown both stimulatory as well as inhibitory effects. It was observed that DL-methionine stimulated laccase production.

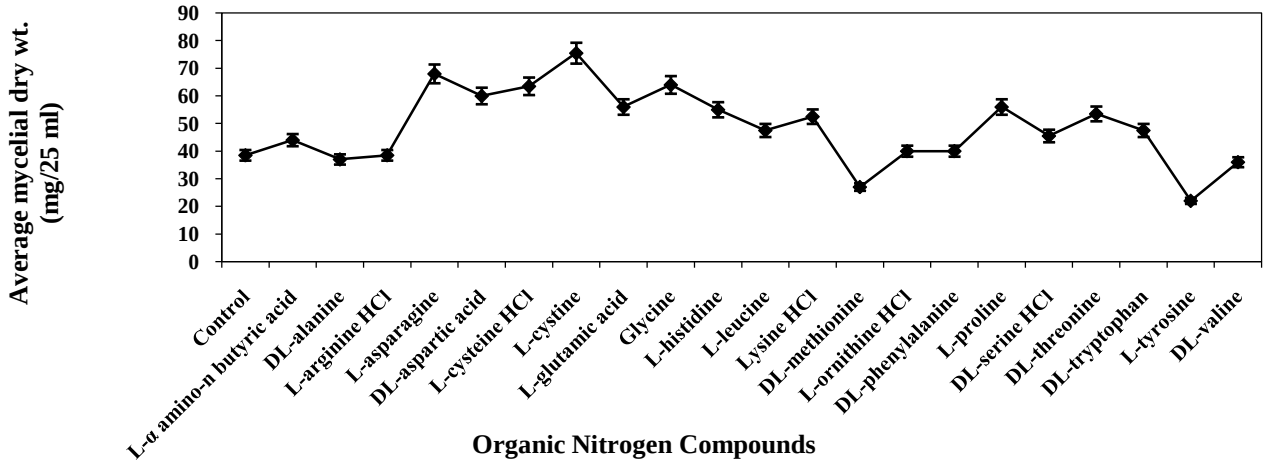

Fig.5. Ligninolytic enzymes production of *Chondrostereum purpureum* in relation to different inorganic nitrogen compounds

Study reveals interesting results regarding growth and ligninolytic enzymes production of the fungus. This data can also be utilized for the mycelial production and ligninolytic enzymes production that can further be used at industrial level for bioremediation of industrial pollutants.

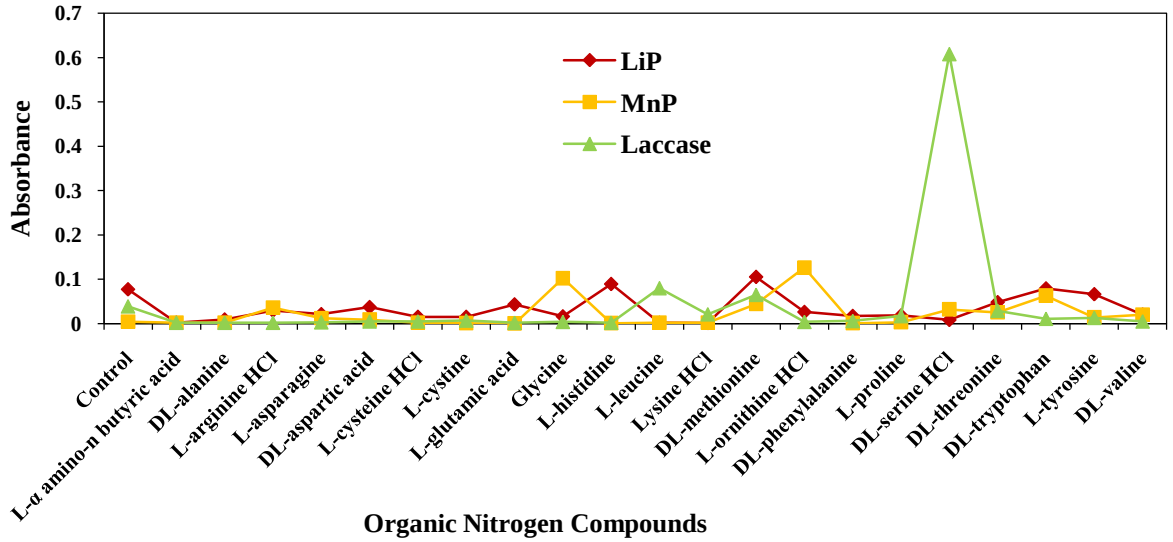

Fig. 6: Ligninolytic enzymes production of *Chondrostereum purpureum* in relation to different organic nitrogen compounds

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