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Effect of Natural Isolates from Mushroom Mycelia on Alpha-Amylase Activity

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Abstract—*α-Amylase and α-glucosidase are two main enzymes involved in carbohydrate metabolism. Many bioactive metabolites have been detected and purified from mushrooms and fungi by several researchers. The present study was aimed at detecting alpha amylase inhibitory activity from edible mushroom mycelia. Edible mushroom was isolated from natural source, from IIT (BHU) campus and was maintained in vitro in mycelial form. Aqueous extraction was performed on 15 days old solid state culture on wheat bran. Water, methanol and acetone were used separately for extraction of active constituent from mycelial cells grown, for 7 days, on potato dextrose broth (PDB). The extracts were tested for alpha-amylase inhibitory activity. The aqueous extract of mycelia grown on wheat bran and methanol and acetone extracts of mycelia grown on PDB were found to have significant alpha amylase inhibition activity. 50% enzyme inhibition was observed by mycelial aqueous extract at a concentration of 0.107ml/ml (grown on wheat bran); and by methanol and acetone extracts at concentration of 0.183ml/ml and 0.109ml/ml (grown on PDB) respectively. Thus the isolated strain may be exploited for production and purification of lead compound for the development of α-amylase inhibitory drug.*

Keywords*: alpha-amylase inhibition, diabetes, white-rot fungi.*

1. INTRODUCTION

A mushroom is the reproductive structure produced by some fungi and is the fleshy, spore-bearing fruiting body of a fungus, typically produced above ground on soil or on its food source. Millions of microscopic spores are formed in the gills or pores underneath the mushroom's cap. Spores germinate to form a network of microscopic rooting threads (mycelium) which penetrate into the food source (wood or soil). Generally, plants make their food using the sun's energy (photosynthesis), while animals eat, then internally digest, their food. Fungi do neither: their mycelium grows into or around the food source, secretes enzymes that digest the food externally, and the mycelium then absorbs the digested nutrients. Mushrooms are one of the oldest forms of life on this planet, pre-dating plants and animals. The largest mushroom currently living is underground in eastern Oregon, ranging 2,000 acres [1].

Mushrooms are distributed worldwide and are consumed by humans since ancient times. The act of consuming mushrooms is called mycophagy. Of the 10,000 known species there are approximately 700 mushroom species that can be eaten as nutritious food [1, 2]. Many mushrooms have also been used for their medicinal properties by old civilizations. The medicinal importance of mushrooms are mentioned in some ancient religious scriptures such as Vedas. Romans considered it as 'foods of gods' and Chinese called it as 'elixir of life' [3]. They have become a very popular valuable food due to their high nutritive value providing many important nutrients including selenium, potassium, riboflavin, niacin, vitamin D, proteins, and fiber [4]. Mushrooms are considered healthy food due to low content of calories, carbohydrates, fat, and sodium, and are also cholesterol free. There are many nutraceutical properties and bioactivities found in a variety of mushrooms, such as antibacterial, antitumor, cholesterol lowering, antioxidant, immune system enhancer and other properties [4]. A general medicinal benefit of consumption of mushrooms is immunity enhancing, which is attributed mainly to polysaccharides (especially β-glucans). Combination of several types of mushrooms in a formula can sometimes create a synergistic immune response. Edible mushrooms having functional properties include *Auricularia, Flammulina, Ganoderma, Grifola, Hericium, Lentinus, Pleurotus*, and *Tremella* [2]. Some medicinal mushrooms such as *Coriolus* are non-edible. A few bioactive molecules have been identified and purified from mushrooms, by several researchers, having alpha-amylase inhibitory activity. For example proteins from *Pleurotus pulmonarius* [5]; lovastatin from *Pleurotus ostreatus* [6] and other bioactive molecules have been identified as potent α-amylase inhibitors.

Edible mushrooms must be organically grown, without the use of fungicides. The metabolite responsible for the medicinal value of the mushroom can be identified, purified and can be used in its pure form as a medicine. The production of potentially health beneficial metabolite(s) by mushrooms are dependent on its genome and expression of genes. If mushrooms are being cultivated for its medicinal value, it is important to make sure that it is genetically same as its wild originals/counterpart. The extraction method is also important as it affects the molecular stability and thus functional property.

One of the main health problems in modern life style is diabetes. The present medication for diabetes is still unsatisfactory in terms of long term effects, despite tremendous research done on this field [7]. Thus finding new types of drugs and novel nutraceuticals for the cure and prevention of diabetes. α-Amylase and α-glucosidase are two main enzymes involved in carbohydrate metabolism. Partially inhibiting the activities of these two enzymes can be helpful in ameliorating diabetes condition [8]. There are many secondary complications in diabetes; one of the most prevalent and fatal complication is increase in the risk of atherosclerosis. Blood pressure decreases in diabetes patient, which slows down the blood flow in arteries, which in turn increases the chance of inflammation. This decreased blood pressure and increasing inflammation may cause atherosclerosis. Atheroclerosis and its symptoms can be reduced indirectly by inhibiting α-amylase enzyme activity in diabetic patients.

2. MATERIALS AND METHODS

2.1 Isolation of fungus

White mature mushrooms were collected from wood logs during the month of august. Sterile water was used for preparing spore water. The mushrooms were soaked overnight in sterile water, and few drops of spores-loaded-water was used for spread plating on solidified potato dextrose agar (PDA) medium. The strain was isolated by sub-culturing by cutting a small piece of agar with the growing edge of the fungus and putting it over another PDA plate. The agar plates were incubated at 28^0C. The hyphal growth was obtained on wheat bran as substrate. The mushroom was also grown in liquid media – potato dextrose broth.

2.2 Extraction (from fungal hyphae grown on wheat bran)

After 9-10 days of growth on wheat bran, 100ml of 20mM phosphate buffer was added to 100g of wheat bran with mycelial growth on it for maceration. The mixture was homogenised using mortar & pestle, and then filtered through cheesecloth. The filtrate was centrifuged at 10,000 rpm for 20 minutes. The clear supernatant was stored at 4^0C and was used for *in-vitro* assays.

2.3 Extraction (from fungal hyphae grown on PDB)

Aqueous, methanol, acetone and chloroform extracts were prepared. Briefly, fungus was grown on PDB, after 7-8 days of growth the cells were separated by centrifugation and the supernatant was stored for analysis. The wet weight of the cell mass was recorded and it was macerated using mortar and pestle. Solvent was added to the homogenised cell mass at approximately 3.5ml/gm (wet cell mass). Sonication was then performed in a water bath for 8 minutes twice. The resulting mixture was soaked overnight in an orbital shaker. It was then centrifuged and the supernatant was filtered was stored at 0^0C and was used for *in-vitro* assays.

2.4 Alpha-amylase inhibition assay

McCue and Shetty [9] procedure was slightly modified and used for the assay [10]. Different amounts of extract (0, 50, 100, 150, 200, 250ul) were incubated with 250ul of enzyme solution (2U/ml) in 20mM sodium phosphate buffer containing 6.7mM sodium chloride (pH 6.9) for 30 minutes at 37^OC. Then 250ul of 1% (weight/volume) soluble starch solution, in the same buffer, was added and the reaction mixture was incubated for 10 minutes at 37^OC. 500ul of DNS reagent was then added to the reaction mixtures and kept in boiling water bath for exactly 5 minutes. Tubes were cooled in running tap water and 5ml distilled water was added to each tube for dilution. The absorbance was measured at 540nm. Controls were prepared for every concentration of extract, without the enzyme. The absorbance values of the controls were subtracted from the experiment values before calculation. Percentage inhibition was calculated by the formula:

$$\%\ inhibition = \left\{\frac{absorbance\ of\ blank\ -\ absorbance\ of\ sample}{absorbance\ of\ blank}\right\} * 100$$

3. RESULTS

3.1 Alpha-amylase inhibition by various extracts

The aqueous extract of the mushroom mycelia grown on wheat bran have significant alpha-amylase inhibitory activity showing almost 100 percent inhibition at a concentration of 0.33ml/ml. 50 percent inhibition was achieved at a concentration of 0.107ml/ml. Methanol and acetone extracts of the mushroom mycelia grown in potato dextrose broth also showed high inhibitory activity with IC 50 values of 183ml/ml and 109ml/ml respectively.

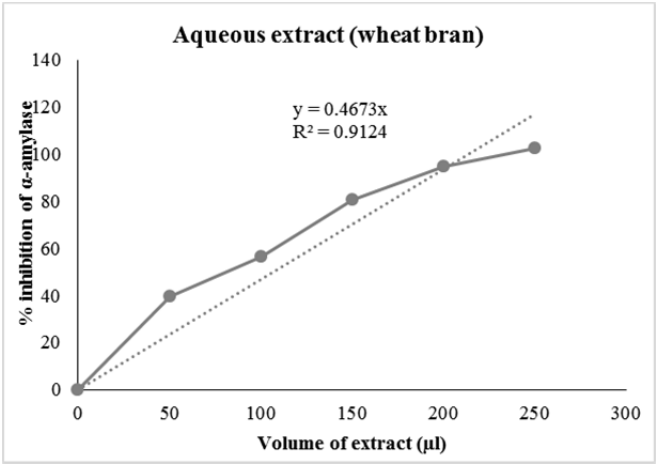

Fig. 1: α-amylase inhibition by aqueous extract of mycelia grown on wheat bran.

The aqueous extract of mycelia grown in PDB had no inhibition activity. To check the enzyme inhibition potential of chloroform extract was not possible as the solvent precipitated the alpha amylase enzyme (protein), and also because of immiscibility of chloroform in water. The liquid media, after removing the cell mass, was also tested for enzyme inhibitory potential and was found to inhibit alpha amylase; showing only 19 percent inhibition at 0.33ml/ml concentration.

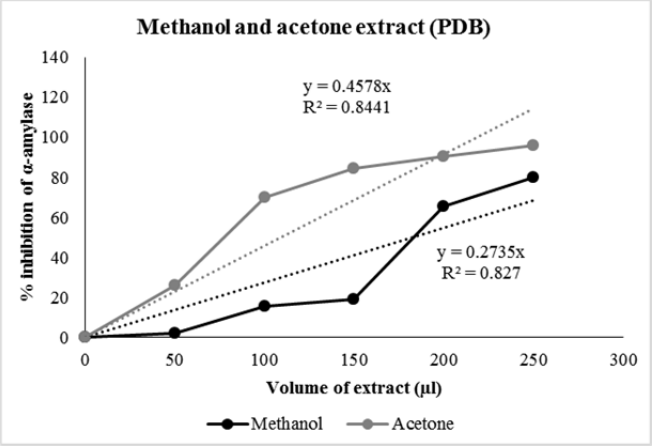

Fig. 2: α-amylase inhibition by methanol and acetone extracts of mycelia grown on PDB.

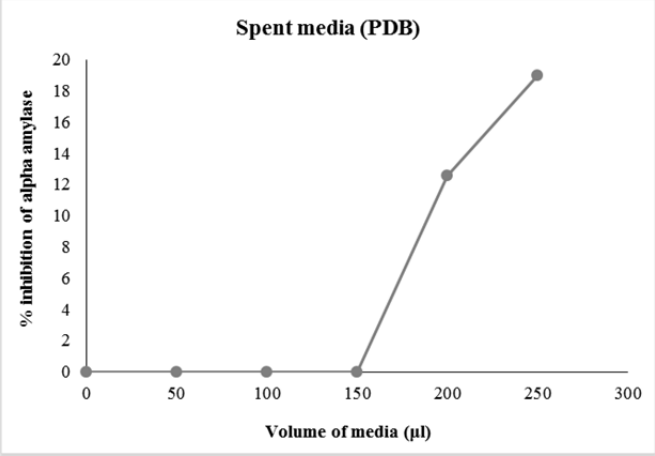

Fig. 3: α-amylase inhibition by potato dextrose medium after separation of cell mass.

4. DISCUSSION AND CONCLUSION

The bioactive constituent(s) involved in alpha amylase inhibitory activity is present both intracellularly and extracellularly since the extracts and the spent medium both are showing inhibition potential. The low inhibitory activity of the spent medium can be attributed to the dilution of the extracellular secretions of the cells in the growth media. Also the enzyme inhibition by aqueous (wheat bran), methanol and acetone extracts can be explained by presence of more than one active constituent or there might be a synergistic effect between different molecules. Further studies are needed for the identification of the bioactive constituent(s). This mushroom species can be exploited for the development of lead compound of alpha amylase inhibitory drug.

5. ACKNOWLEDGEMENTS

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