African Journal of Biotechnology Vol. 10(69), pp. 15578-15586, 7 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.685
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Evaluation of nutritional substrate and physical stress (gamma irradiation) in β-glucan productivity by mushroom (*Pleurotus ostreatus*)

Mohamed E. A. Dawoud[1]* and Amira M. Abu Taleb[2]

Botany Department, Faculty of Science, Cairo University, Egypt.

Accepted 12 October, 2011

β-Glucans present in mushrooms are bioactive polysaccharides that have many pharmacological (immuno-modulatory, antitumoral, hepatoprotector, anti- inflammatory and antioxidant) activities. The β-glucans productivity of the mushroom depends upon the growing conditions, nutritional substrates, biotic and abiotic stress factors that result in an enzymes over-expression. The study shows the potential response of glucan production by fungi grown on newly designed animal plant crude (hydrolyzate) extract broth (APCEB) substrate (animal-, plant-waste crude extract broth fortified with 1g KH_2PO_4 l^{-1}) and gamma irradiation. On application of physical stress factors (gamma irradiation, 1 to 6 kGy), 1 kGy gamma irradiation doses resulted in an increase in: protein (37.5%), carbohydrates (32.15%), glucans (12.0%) and growth (dry mass, 14.8%); these were due to the enhancement of low doses (1.0 kGy) of gamma irradiation upon growth and metabolism that was synergized by the radioprotective components (cysteine) that prevailed in the animal extract. This substrate supported the growth and glucan production of *Pleurotus ostreatus* than by *Schizophyllum commune* and *Saccharomyces cerevisae*. There are suggested procedures to assure the percentage of glucan purity and entity. The APCEB substrate is a general basic formula in which the animal and plant remains can be replaced by many others. The yield of this media economizes the glucan production.

Key words: β-Glucans, *Pleurotus ostreatus, Schizophyllum commune, Saccharomyces cerevisae, gamma* irradiation, submerged cultures and nutritional synergism.

INTRODUCTION

Commercial importance of fungal polysaccharides has attracted much attention in the field of functional foods. Partially, commonly cultivated mushroom of the *Genus Pleurotus* are interesting because of its β-glucans that demonstrate great immunomodulation, antioxidant, anti-inflammatory and analgesic properties (Smiderlie et al., 2008). Moreover, the immunological, pharmaceutical, and nutraceutical characteristics of β-glucans were clearly elucidated by several authors where, Richel et al. (2011) recorded that energetic food containing β-1,3 and 1, 6-D-glucan from Oyster mushroom resulted in more favorable (*P<0.01*) health and immune indices in high yielding dairy cows. Lavi et al. (2010) stated that mushroom poly-saccharides are potent substances that exhibit anti-tumor and immunomodulatory properties. A high level of biological efficiency has been found in β-glucans, especially β-1,3-D glucans and β-1,6-D glucans isolated from some basidiomycetes (biological efficiency refers to the relative ability of β-glucans to promote a desired

*Corresponding author. E-mail: mez_dawoud@yahoo.com. Tel: 0106975447 or 0233825405.

Abbreviations: APCEB, Animal plant crude (hydrolyzate) extract broth; **MYA,** malt yeast extract glucose agar; **CE,** crude hydrolyzate extract; **ACE,** animal crude extract; **NCRRT,** national center for radiation research and technology; **DRV,** direct reducing value; **GHL,** glycerol content of hydrolyzed lipid; **BOD,** biological oxygen demand.

response, for example to induce leukocyte activation and to produce inflammatory mediators). Angeli et al. (2009) found that, β-glucan extracted from medicinal mushroom (*Agaricus blazei*) has a chemoprotective effect that prevents the genotoxic effects of benzo (a) pyrene in human hepatoma cell line Hep G2. Shen et al. (2007) suggested that, smaller amounts of micellary β -glucan might be useful for the potentiation of intestinal immunity. Mantovani et al. (2008) stated that the polysaccharides β -glucans substances stimulate the immune system, modulating humoral and cellular immunity, and thereby have beneficial effect in fighting infections (bacterial, viral, fungal and parasitic). β-Glucans also exhibit hypocholestrolemic and anticoagulant properties, they have been demonstrated to be anticytotoxic, antimutagenic and antitumorogenic making them promising candidate as pharmacological promoters of health.

There is a lot of different species in *G. Pleurotus* that have pharmacological properties for example *Pleurotus florida, Pleurotus tuber-regium, Pleurotus pulmonarius, Pleurotus ostreatus* and *Pleurotus eryngii* (Ragunathan et al., 1996). The dry matter of mushroom fruit bodies is about 5 to 15% of its wet mass; they have very low fat content and its contain 19 to 35% proteins. Mushroom fruit bodies have plenty of vitamins, mainly B_1, B_2, C and D_2 (Manzi et al., 2004). From these mushrooms, several different kinds of polysaccharides have been produced from liquid culture, fruit bodies and their physiological activities have been elucidated. However, their applications have been concentrated on mainly medicinal uses such as antitumor and immunostimulating agents (Kim et al., 2001). The content of these carbohydrates, which are mainly present as polysaccharides or glycoproteins, ranges between 50 to 90%. Most abundant mushroom polysaccharides are chitin, hemicellulose, β- and α-glucans, mannans, xylans and galactans. The average molecular mass (MW) varies according to the source and ranges from 5 to 2000 kDa (Bohn and BeMiller, 1995). Mushroom polysaccharides are present mostly as linear and branched glucans with different types of glycosidic linkages, such as (1→3), (1→6)-β-glucans and (1→3)-α-glucans, but some are true heteroglycans containing glucuronic acid, xylose, galactose, mannose, arabinose or ribose (Wasser, 2002). Other microorganisms produce β-glucans which is used as a component of various cosmetics. It is mainly produced from *S. cerevisiae* as water-soluble particular or it's chemically modified soluble form such as carboxylmethyl or phosphorylate glucan (Czop and Kay, 1991). Another β-glucan, schizophyllan, which is produced from *Schizophyllum commune*, has been used as an immunotherapeutic agent for cancer treatment in Japan since 1986, because it increases body immune defense mechanism, (Yanaki et al., 1983).

However, it has been difficulties to use schizophyllan in cosmetics due to its high production cost (Yanaki et al., 1981). Moreover, Borchers et al. (2004), stated that, β-glucan is composed of glucose units linked together to form a long polymer chain and is a fiber type derived from the cell wall of yeast, oat, barley and many medicinal mushroom. It was stated that, to date, mushroom are one of the largest untapped source of powerful and new pharmaceutical product (Wasser, 2002). The author added that polysaccharides from mycelia and its culture media have also been found to have immunomodulatory and antitumor activities that are comparable with the fruit bodies. Concerning the interrelation between irradiation and glucan, Hugo (1995) irradiated the glucan product with γ-rays and concluded that, gamma irradiation could be used as an effective method for the production of depolymerized β-glucan,and improve functional properties such as immunomodulatory activity. Moreover, physical stress factors play a major role in mutation, where they are used to improve the biotechnological and biological performance of poorly characterized strains of microorganism. Meng et al. (2007) developed a method using ultraviolet (UV) irradiation for mutation and developed an improved strain of *Acidianus brierleyi* with a good tolerance of Cu and four fold bioleaching rate compared with bob-treated strains. The first step in our investigations was to design and select an over-productive artificial microbiological medium and to evaluate its potential on biomass, polysaccharides (glucans) and other metabolites production, and then the application of gamma irradiation techniques to study their roles on growth, metabolism enhancement and metabolite synthesis. The researches neither emphasize the role of physical stress factors (irradiation), different fungal radio-tolerance (during β-glucans production) nor the difference in potentiality of various fungi. So this investigation aimed at designing new potentially over-producing fungal substrate and estimation of its chemical composition, then application of stress factor techniques of gamma irradiation (1 to 6 kGy), and finally selection of the promising radiotolerant, fast growing- and glucan over producing fungus for further studies.

MATERIALS AND METHODS

Microorganisms used

A culture of *P. ostreatus* (from our previous lab collections), *S. Cerevisae* and *S. commune* were provided by Agricultural Research Center, Giza, ARE. The stock cultures were maintained on malt yeast extract glucose agar (MYA) slants. Slants were incubated at 28°C for seven days and then stored at 4°C till use (Dawoud and Eweis, 2006).

Preparation of the newly designed fungal medium (substrate)

It was formulated from waste products of animals (Poultry hind limb, A), plants (waste grape fruits, P) and fortified with 1 g KH_2PO_4 l^{-1}: 100 g, of slaughter waste poultry hind limbs (A) were dried (60°C), ground and acid (100 ml 6 N HCl) hydrolyzed at 80°C for 24 h, (Dufosse et al., 1997) that gave 80% acid crude hydrolyzate extract (CE) which was neutralized with 10 N NaOH (to pH 7), and filtered completely to 1L with deionized water. The final clear filterate was termed as animal crude hydrolyzate extract (ACE). 2% (v/v) of ACE

was found to be the optimal concentration for fungal growth. ACE was fortified with 1 g KH_2 PO_4 and 20 g glucose equivalent sterile waste grapes juice so the substrate was formulated as: 20 ml of ACE, 20 g equivalent grapes juice, 1g KH_2 PO_4, 1 L deionized water and called APCEB. It must be taken into consideration that, this medium is a general basic formula (that is, A can be constituted from other animal and plant wastes that is, poultry for limbs, viscera, heads, feathers; B, can be replaced by any waste fruits).

Growth conditions and stress factors application

Growth conditions

Two sets of flasks, for each fungus, one containing 100 ml of APCEB and an other containing modified MYB (for comparisons), (pH 5.5) were distributed in 500 ml flasks, autoclaved for 15 m at 15 lb/ sq inch, 121°C and inoculated with previously irradiated [1 to 6 kilo Gray (kGy)] fungal discs (5 mm diameter). The fermentation period was seven days at 28°C. After the incubation period, the cultures were filtered thoroughly, and the mycelium was washed. Parts were used for fresh mass record and other for mycelial extraction, the others were dried at 60°C until constant weight (the dry masses were recorded) and kept for further chemical analysis.

Stress factors application

For irradiation experiment discs, 0.5 cm diameter of seven days old culture of *P. ostreatus, S. cerevisae and S. commune* were raised on sterilized Petri-dishes containing modified MYA medium. After incubation for seven days at 28°C, cultures were subjected to doses of 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 (kGy). Irradiation was carried out in Co^{60} gamma cell located at National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo. The gamma source gave a dose of 25 Gy/min at the time of experiment. Different irradiated discs were inoculated in the above mentioned flasks (500 ml capacity).

Carbohydrate analysis

Mycelial (and substrate) extraction

Fresh fungal mycelial samples (or APCEB substrate) were homogenized (or mixed) in known volume of borate buffer at pH 8. The homogenate was transferred to small tubes and kept standing over night before centrifugation. The residue was dried at 80°C (used for determination of total polysaccharides) while the filtrate was made up to 15 ml volume and used for nitrogen and soluble phosphorus compounds. The value of glucan was subtracted from total polysaccharide to give the non-glucan polysaccharides; the values of total polysaccharides + (mono-, disaccharides) = total carbohydrates. Equal volumes of the tissue extract and 15% trichloroacetic acid were mixed to ensure precipitation of soluble peptides and then centrifuged to eliminate the interference of this nitrogen component during chemical analysis of carbohydrate.

Direct reducing value (DRV) of substrates

Direct reducing value (including all free monosaccharides and hydrolysis product of disaccharides) was carried out by evaporation of 0.1 ml of cleared borate buffer extract till dryness and then mixed with 1 ml of the modified Nelson solution (Said and Naguib, 1964). The mixture was left on a boiling water bath for 15 min. Thereafter, it was cooled rapidly using running tap water, then 1 ml of arsenomolybdate was added according to the modified method of Nelson (Dawoud, 1986). The mixture was diluted to definite volume, and the developed color was measured spectrophotometrically at 700 nm using Berkin Elmer 35 spectrophotometer, UK.

Total polysaccharides

A definite weight of the fungal mycelia was introduced into a test tube, to which 10 ml 1N HCl were added and hydrolyzed by heating in a boiling water bath for at least 2 h. The extract was then filtered, neutralized, made up to volume and finally the reducing power was estimated as mentioned above.

Isolation and purification of *β-glucan* polysaccharides

The fungal mycelia were washed with 80% (w/w) ethanol then washed with distilled water and extracted with boiling water for 6 h; extracts were incubated with α-amylase at pH 7 for 30 min to remove α-glucans. Sevag reagent (chloroform/butanol 4:1, v/v) was applied for deproteinization then the deproteinized supernatant were dialized and lyophilized to give water soluble fractions. The insoluble parts were extracted with 1M solution of sodium hydroxide containing 0.05% of sodium borohydride, the solid part was assigned as insoluble fractions and the supernatant was adjusted in the same way (as previous one) to give the alkali soluble fractions. Boiling water and alkali extracts were additionally treated with phenolic to remove the remaining protein (Synytsya et al., 2009). The different glucan fractions were collected and were assigned as total glucan.

Test for glucan entity and percentage purity

A known weight of the obtained glucan was hydrolyzed by glucan hydrolase enzyme and the amount of hydrolysis product (glucose weight) was calculated and matched with the original weight. When the two values are identical this indicates 100% purity.

Protein analysis

Protein content in the borate buffer extract and the residual mat (1NaOH extract) was determined according to the method of Lowry et al. (1951).

Amino acid analysis

Separation and estimation of free amino acid pool in the ACE substrate was determined using LC 3000 amino acid analyzer. The method adopted was that of Razak (1980).

Determination of elements in the substrate

The samples (known volumes or weights) were digested with a mixture solution of concentrated H_2SO_4-HNO_3 (1:1 v/v). The digestion was carried out at 180 to 200°C on a sand bath until the dense white fumes of H_2SO_4 were evolved. The digest was cooled and 0.5 ml $HClO_4$ was added. The digestion was continued until the acid liquid was largely volatilized, and then the remaining dense solution was diluted to a known volume by distilled water. This stock solution was used for estimation of Na, K, Ca, Mg and P was determined photometrically by using flame photometer (Allen et al., 1974). Magnesium, phosphorus, manganese, zinc, ferrous and copper contents were determined using atomic absorption spectrophotometer (UV/HS-360, Germany). Chloride content was determined by titration with standard $AgNO_3$; 1ml $AgNO_3$ 0.01N is

equivalent to 0.000335 g Cl (Ward and Johnson, 1962). Analytical grade chemicals were used in the experiments. All reported results are the average of, at least, three determinations.

Glycerol analysis

Free glycerol was extracted from the dried powdered mycelia with boiling water and estimated in the clear water extract as performed by Younis et al. (1987).

Lipid analysis

Extraction of lipid followed the procedure outlined by Viladomat et al. (1986). The extracted lipid was subjected to alkaline hydrolysis using Schmidt-Thannhouser procedure described by Clark and Switzer (1977). The content of liberated glycerol was determined according to the procedure described by Younis et al. (1987). The obtained value was considered as the glycerol content of hydrolyzed lipid (GHL). Glycerol + lipid content give the value of fats.

Yield measurements (Dawoud and Eweis, 2006)

$$\text{Biological efficiency (B.E)} = \frac{\text{Fresh weight (g)}}{\text{Dry weight of substrate (g)}} \times 100$$

It is the relative potentiality of substrate to induce desired productivity in dry mass gain.

Total energy (k cal) = 4 (g protein + g carbohydrates) + 9 (g lipids)

Statistical analysis

The experiment followed complete randomized design. The obtained data were subjected to analysis of variance (ANOVA) according to Snedcor and Cochran (1980) using Mastate programme. The least significant differences were used to compare means of treatments according to the Walter and Duncan (1969) or probability of 5%.

RESULTS

The main chemical constitution of APCEB and MYB media is shown in Table (1). The data show that both media contain all organic and inorganic materials required for microbial media but APCEB was superior to MYB in the major carbon (total sugar content 5.5 times), nitrogen (10.0 times; protein, 11.01 times) and mineral elements (3.6 times) but amino acids content was nearly equal in quantity. Glutamic, glycine, and aspartic were relatively higher in amount in both media but methionine and cysteine were relatively higher in APCEB medium, proline and tryptophan were mostly absent from both media. The higher quantity of mineral elements was due to the higher abundance of K and P followed by Ca, Mg and Na that represent the large proportions (the whole sum represents 86% of the total mineral elements). In determination of the optimum ACE for fungal growth, it was found that 2% was the optimum that produced the highest biomass yield (Table 2). Higher and lower concentrations decreased the growth (g. d. Mass. l^{-1}). The productivity of APCEB compared with MYB medium under gamma irradiation stress (Table 3) showed that, the mean and percentage dry masses of the test fungi were relatively higher in the case of APCEB than in MYB medium. On the other hand, the radio- tolerance (represented as g d. mass l^{-1}) of test fungi was higher among the *P. ostreatus* than *S. cerevisae* and *S. commune.* Furthermore, 1 kGy dose of gamma irradiation enhanced the growth of *P. ostreatus;* this led us to select the APCEB medium for further experiments. The data (Table 4) further showed that, APCEP medium supported higher production of fresh dry masses, glucan, polysaccharides, total carbohydrates, protein and fats and their subsequent interrelation and ratios by *P. ostreatus* under the effect of gamma irradiation stress. The most important finding was the increase in the biological efficiency and total energy of ABCE production over MYB media.

DISCUSSION

We selected some fungi of wide biotechnological application: *S. cerevisae, S. commune* and *P. ostreatus.* These fungi, specially the latter, have agricultural and biotechnological values, it thrive on wide varieties of lignocellulosic, animal remain substrates and is known for its ease cultivation and high adaptability (Gregori et al., 2007). Of these fungi, *P. ostreatus* mushroom was selected on the basis of its high biomass productivity and its radiotolerant character (Table 3) that varies from one individual to another and between organs of the same individual (Prasad, 1995). In addition, utilization of animal remains decrease environmental pollution and provide rich nitrogenous substrates that increase the proteomic components of the growing fungi. Sher et al. (2011) stated that mushroom production can play an important role in managing farm organic wastes when agricultural and foods processing by product are used as a growing media for edible fungi. Moreover, it was stated that the type of metabolic product and its biological activity depend upon the constitution of the medium. In this concern, Paulik et al. (1996) reported that *P. ostreatus* mushroom cultivated on the date wastes possessed a potent antitumor activity against Thrlich ascites carcinoma. Carboxymethylated glucan from *P. ostreatus* exhibited immunomodulatory effect, especially enhanced phagocytic activity.

The composition of the medium may affect the enzyme expression. Ahlawat et al. (2008) stated that, the strains of mushroom (*Volvariella volvacea*) with low intensity protein bands, in its profile, also varied in mycelial growth rate on two different media, possessed high activity of endoglucanase, β-glucosidase, laccase and polyphenol oxidase. Abo Shady et al. (2000) found that corn steep

Table 1. Main chemical composition of APCEB and MYB broth.

Major component (mg 100 ml^{-1} medium)	APCEB	MYB	Mineral element (mg 100 ml^{-1} medium)	APCEB	MYB	Amino acid (mg 100 ml^{-1} medium)	APCEB	MYB
Nitrogen	200 ± 8.90= 14×10^{-4} ± 0.001 M	22.0±0.71	Na	3.0 ± 0.10	0.50 ± 0.02	Glutamic	18 ± 0.70	10.4 ± 0.40
Protein	110 ± 5.0	10 ± 0.35	K	27.0 ± 0.80	1.5 ± 0.005	Glycine	13.2 ± 0.50	10.1 ± 0.40
Total sugars	22×10^{2} ± 68.11	1400 ± 40.0	Ca	5.0 ± 0.20	1.5 ± 0.005	Aspartic	8.0 ± 0.30	16.0 ± 0.60
Total lipids	6.0 ± 0.2	4 ± 0.15	Mg	4.0 ± 0.20	2.4 ± 0.01	Serine	6.0 ± 0.30	7.0 ± 0.25
			Mn	0.6 ± 0.10	0.18 ± 0.04	Threonine	4.2 ± 0.20	5.0 ± 0.20
			Zn	1.2 ± 0.05	0.06 ± 0.005	Alanine	7.0 ± 0.29	5.5 ± 0.20
			Cu	3.0 ± 0.10	0.18 ± 0.05	Leucine	9.0 ± 0.30	9.5 ± 0.4
			Fe	2.8 ± 0.10	1.5 ± 0.005	Valine	6.0 ± 0.28	7.0 ± 0.25
			P	22.0 ± 0.70	9.0 ± 0.30	Lysine	5.0 ± 0.2	6.1 ± 0.20
			Cl	2.7 ± 0.10	3.0 ± 0.11	Arginine	10.0 ± 0.4	8.0 ± 0.25
						Proline	-	0.1 ± 0.01
						Phenyl Alanine	3.8 ± 0.15	4.8 ± 0.20
						Tyrosine	3.6 ± 0.15	4.0 ± 0.20
						Isoleucine	4.0 ± 0.20	3.0 ± 0.20
						Methionine	10 ± 0.40	1.0 ± 0.10
						Cysteine	5.0 ± 0.20	3.2 ± 0.10
						Tryptophan	-	-
			Total	71.3 ± 2.10	19.82 ± 0.40	Total	103.8 ± 3.30	101.6 ± 3.03

Table 2. Effect of different concentrations of animal crude extract (ACE, ml/ 100 ml^{1}) on growth (g d. mass l-1) of *Pleurotus ostreatus*, *Saccharomyces cerevisae* and *Schizophyllum commune.*

Dry mass (g l^{-1})	0.0%	1.0%	2.0%	3.0%	4.0%	5.0%	6.0%	7.0%	80%
Pleurotus ostreatus	0.50±0.05	2.00±0.1	9.90±0.80	7.0±0.60	4.90±0.3	4.0±0.3	3.00±0.2	1.0±0.1	-
Saccharomyces cerevisae	0.60±0.05	1.60±0.09	7.00±0.60	4.9±0.50	3.0±0.2	2.8±0.2	2.70±0.2	0.8±0.1	-
Schizophyllum commune	0.40±0.03	1.90±0.1	7.20±0.60	5.00±0.5	3.2±0.3	2.0±0.10	1.50±0.1	-	-

liquor and potato waste were the best nitrogen source that supported single cell protein by *Candida utilis*, *Candida tropicalis,* and *Schwanniomyces occidentalis.* Hussein et al. (2002) tried black liquor hemicelluloses of rice hull for the production of cellular protein by Aspergillus *ustus,* and *Aspergillus terreus* and found that high biomass yield was obtained by *A. ustus.* It is worth noting that the composition of media not only affect the metabolic content of the growing fungus but also the character of product where it was found that β -glucan (from *S. cerevisae*) gave10% increase in collagen biosynthesis (Kim et al., 2000). Furthermore, Kosaric and Miyala (1981) reported that protein content varies depending on the substrate and *Morchella hybridia* contains only 10.5% protein when grown in synthetic glucose medium, but 34.8 and 37.5% when grown in

Table 3. Quantification and percentage of biomass, protein, polysaccharides, total carbohydrates, β-glucans, fats, biological efficiency, substrate dry weight and total energy content of *Pleurotus ostreatus* growing on APCEB/MYB media under the effect of 1 kGy gamma irradiation dose.

Substrate	MYB medium						APCEB medium					
	P. ostreatus		*S. cerevisae*		*S. commune*		*P. ostreatus*		*S. cerevisae*		*S. commune*	
γ -Irradiation dose (kGy)	M	%	M	%	M	%	M	%	M	%	M	%
0.0	8.92^{A}±0.35	100.0^{A}±6.20	5.82^{A}±0.22	100.0^{A}±5.90	5.99^{A}±0.21	100^{A} ±5.2	10.24^{B}±0.42	100.0^{B}±3.99	6.52^{A}±0.29	100^{A} ±4.11	6.81^{A} ±0.3	100^{A} ±5.0
1.0	9.01^{A}±0.40	101.00^{A}±6.50	3.02^{B}±0.19	62.65^{B}±3.32	5.02^{B}±0.21	83.81^{B}±3.61	11.52^{A}±0.45	109.38^{A}±3.2	5.61^{B}±0.28	86.04^{B} ±3.91	5.82^{B} ±0.25	85.46^{B}±3.91
2.0	7.00^{B}±0.21	78.48^{B}±3.99	2.98^{B}±0.18	61.83^{B}±3.39	4.32^{C}±0.19	72.12^{C}±3.00	9.02^{C}±0.40	88.09^{C}±3.22	4.01^{C}±0.20	61.50^{C} ±3.2	4.00^{C} ±0.2	58.74^{C}±3.0
3.0	5.42^{C}±0.19	60.76^{C}±3.01	2.50^{B}±0.15	50.87^{C}±2.62	3.50^{D}±0.18	58.43^{D}±2.51	6.11^{D}±0.31	59.66^{D}±2.91	3.60^{D}±0.15	55.21^{D} ±2.5	3.81^{D} ±0.15	55.95^{D}±2.11
4.0	4.11^{D}±0.39	46.08^{D}±2.52	1.59^{C}±0.10	32.99^{D}±1.81	2.46^{E}±0.15	41.07^{E}±2.00	4.92^{E}±0.29	48.05^{E}±2.01	3.01^{E}±0.15	46.01^{E} ±2.11	3.01^{E} ±0.12	44.05^{E}±1.98
5.0	-	-	-	-	-	-	-	-	-	-	-	-
6.0	-	-	-	-	-	-	-	-	-	-	-	-
Main effect of γ -irradiation (2.0)	6.89^{B}±0.39	77.26^{B}±2.56	2.99^{B}±0.18	61.67^{B}±2.56	4.26^{C} ±21	71.09^{C}±3.01	8.36^{C}±0.39	81.04^{C}±3.2	4.55^{C}±0.28	69.75^{BC}±3.51	4.69^{BC}±0.27	68.84^{BC}±2.29

Table 4. Growth response of *Pleurotus ostreatus, Saccharamyces cerevisae and Schizophyllum commune* (g d. mass l^{-1}) to APCEB* and MYB** media and gamma irradiation dose (1 to 6 kGy).

Fungal metabolites and efficiency (g l^{-1})	Medium (substrate)		% APCEB/MYB
	MYB	APCEB	
Fresh mass	79.00±4.2	102.00±4.5	129.11
β-glucans	4.03±0.15	6.19±0.32	112.20
Dry mass	8.00±0.35	10.0±0.40	125.00
β-Glucans/dray mass	45.18±2.21	60.45±3.11	133.80
Proteins	1.60±0.03	2.2±0.05	137.50
β-Glucans/proteins	251.88±17.9	313.64±19.0	124.50
Polysaccharides	6.22±0.20	8.03±0.49	129.10
β-Glucans/polysaccharides	64.79±4.11	77.09±4.2	141.88
Total carbohydrates	6.50±0.22	8.59±0.50	132.15
β-Glucans/carbohydrates	62.00±3.0	72.06±3.5	116.23
Proteins/dry mass	179.37±10.1	214.84±11.90	119.77
Polysaccharides/dry mass	79.73±3.32	78.42±0.3	98.35
Total carbohydrates/dry mass	6.50±0.22	8.59±0.50	132.15
Substrate (medium) Weight/l	20 g malt, 0.5 dried yeast extract Total = 20.5	20 gm glucose, 1g KH_2PO_4 0.2 dried animal extract Total + 21.2	103.4
Fats	0.2±0.001	0.22±0.001	110.00±3.90
Biological efficiency	385.37±14.32	481.132±15.9	124.85±4.22
Total energy content (k. cal)	34.200±1.99	43.16±2.59	126.20±4.35

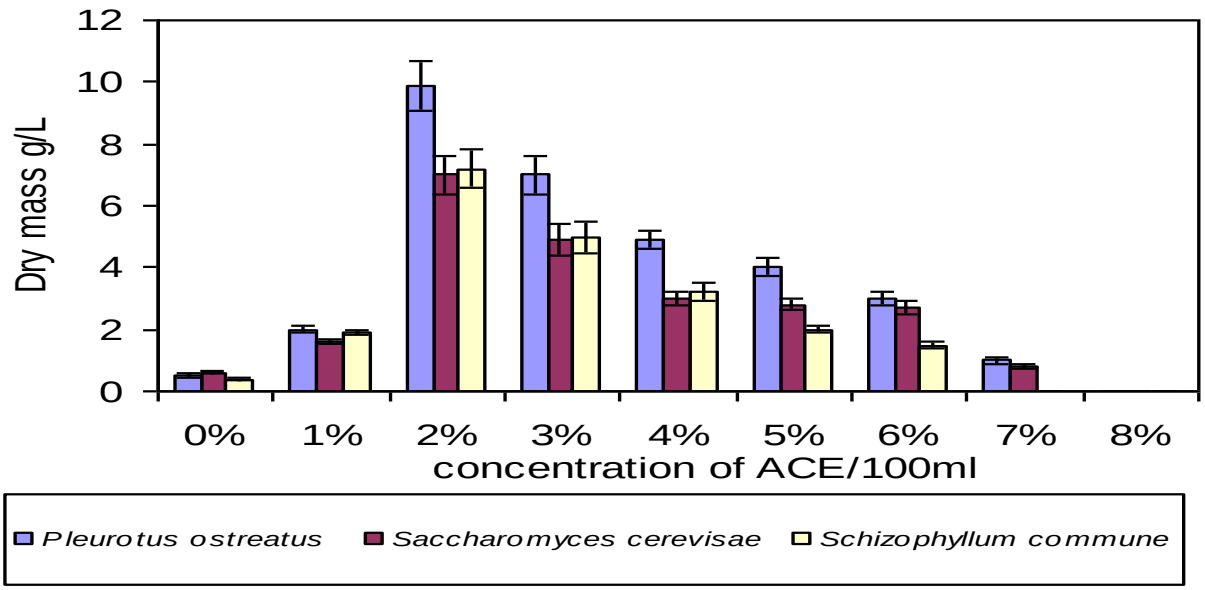

Figure 1. Effect of different concentrations of animal crude extract (ml/ 100 ml^{-1}) on growth (g d. mass. l^{-1}) of *Pleurotus ostreatus*, *Saccharomyces cerevisae* and *Schizophyllum commune*.

molasses and waste sulphite liquors, respectively. In the determination of the optimum concentration of poultry limb hydrolysate (ACE) (Table 2 and Figure 1), it was found that 2% was the optimum while 3% decreased the growth (dry mass). The inhibitory effect (at higher and lower concentration) may be due to high biological oxygen demand (BOD), load of ACE, presence of cell wall cations and some toxic materials. Similar effect has been observed from effluent with high load of organic and inorganic materials (Kadioglu and Algur, 1992).

Thus, 2% ingredient of ACE was termed as ACE and used as a constituent of APCEB medium in all experiments. APCEB contains 20 g glucose equivalent waste sterile grapes juice, 20 ml ACE, and 1 g KH_2 PO_4 per liter. The amount (50 g) of poultry hind limbs was hydrolyzed into 40 g hydrolysis product, accordingly 80% of poultry limb (hydrolysate) can be evaluated as a substrate source in the production of *Pleurotus* and its active metabolites. The stimulatory effect of APCEB production over MYB medium in biomass yield (Table 3), carbohydrate synthesis, percentage glucan and other metabolite production (Table 4) might be due to the abundance of the substance produced from acid hydrolysis, such as carbon, nitrogen sources, and mineral elements which are abundant in APCEB compared with MYB, amino acid content, sulfur-containing amino acids that suffice the growth of organisms (Table 1). Mineral elements play a major roles in metabolism; for example, Mg has an essential function as bridging element for aggregation of ribosome subunits (Cammarano et al., 1972) a process that is necessary for protein synthesis. Moreover, the chemical composition of the newly designed APCEB is in accordance with the findings obtained from the investigation on the elemental and amino acid composition of various animal and plant wastes (Dufosse et al., 1997). On the other hand, the impact of γ-irradiation was elucidated by several studies which showed that many radio-protective substances (cysteine that is available in animal tissue) when present in the culture media protect the irradiated microorganisms from radiation damage at all stages of the cell cycle (Dewey, 1960). The radioprotective substances improve the radioresistance character of the microorganisms adapting them to alteration.

In this connection, Craig and Alt (2004) stated that, the cellular response to single and double strand deoxyribonucleic acid (DNA) breaks include arrest or progression in cell cycle, check points and induction of DNA repair. Unrepaired or misrepaired DNA damage can result in genetic or genomic instability. Eucaryotic cells have evolved efficient mechanism to detect and repair DNA lesions induced within each cell phase of the cell cycle. Furthermore, the increase in the percentage biomass/APCEB was in part, due to the increase in metabolic products of different fungi especially under 1 kGy gamma irradiation recording 109.38, 86.04, and 85.46 against 101, 62.65 and 83.81% for *P. ostreatus*, *S. cerevisae* and *S. commune* on APCEB against MYB medium, respectively. This may point to the abundance of radio-protective substances in the composition of the APCEB medium. The mechanisms of these radiation protections include: hypoxia (removal of molecular oxygen from the solution which reduce radiation damage), free radical scavenger, mixed disulphide mechanism, plysiological shock mechanism and reversible DNA synthesis (Facq and Alexander, 1961).

El-Hifnawi (2002) found that, significant growth stimulation occurred at a dose of 0.3 kGy and the response to single dose may be dose dependent, the result showed that radiostimulation changed the antibiotic sensitivity of the *Staphylococcus aureus* NCRRT to different antimicrobial agents.

In our experiment, the failure of growth of the test fungi to higher gamma irradiation (5.0, 6.0 kGy) was due to the sensitivity (intolerance) to the impact of higher doses that affect all cellular organelles (cytoplasm mitochondria, ribosomes, glogi apparatus and plasma membranes) macromolecules (DNA, ribonucleic acid (RNA), Enzymes). In this concern, Prasad (1995) proposed that the radiosensitivity (death or inhibition) of cells is directly proportional to the DNA content of different species (due to different energy absorption by DNA molecules), and impairment of radio resistant process, which is an individual character. In addition (and after impairment of radio-resistant mechanism), cytoplasmic changes after irradiation include swelling, vacuolization, disintegration of mitochondria and endoplasmic reticulum, changes in the golgi apparatus and polysomes, and nuclear changes (swelling of the nuclear membrane and disruption of chromatin materials). El-Sayed et al. (2001) stated that, gamma irradiation inhibited the growth of *P. italicum* and *A. flavus* at a dose of 2.5 kGy and the growth decreased to 50%. In general, X-and γ-rays produce modifiable damage primarily by free radicals; α- particles and proton produce non modifiable damage primarily by ionization. The observed decrease in biomass, of the test fungi, with rise of dose of gamma irradiation, was due to the inverse relation between irradiation doses, rate of growth and metabolism.

The difference in sensitivity and subsequent meta-bolism toward irradiation are individual organism dependent. In this respect, El-Mongy et al. (2001) found that there was an increase in protein production by *Zygosaccharomyces roxii* and *S. cerevisae* exposed to 0.25 kGy when compared with non irradiated control. Meanwhile there was no increase in case of *Candida tropicalis* and *Schwanniomyces occidentalis* exposed to the same irradiation doses. The D_{10} value of the tested yeast isolates were calculated and found to be 1.85 kGy for *S. cerevisae*, 1.58 kGy for *S. occidentalis,* 1.31 kGy for *C.tropicalis* and 1.22 kGy for *Z. roxii*, higher irradiation (Sublethal) doses levels of 6.0 and 6.25 kGy caused decrease in growth of *Z. rouxii*. Bashandy and Hifnawi (2002) found that spore forming bacilli are highly radioresistant when exposed to gamma-irradiation at low dose rate 1.24 kGy than high dose rate; 6.70 kGy/h (no growth) and conversely, the stimulatory effect of 1.0 kGy gamma irradiation on dry mass due to activation of cellular and extracellular metabolic enzymes.

Conclusions

Stimulation of *P. ostreatus* growth (on APCEB medium) by 1 kGy gamma irradiation resulted in an increased meta-bolism, best glucan synthesis, higher biological efficiency and energy content than other experimental conditions (non irradiation, other test fungi and MYB medium) did.

REFERENCES

Abo-Shady MR, Hammad AAI, El-Mongy TM, Taha SM (2000). Incidence Toxigenicity and control of *Aeromonas* species in different Egyptian food products. Az. J. Microbiol. 47: 62-73.

Ahlawat OP, Gupta P, Komal S, Dahr BL (2008). Development of molecular and biochemical markers for selecting a potential high yielding strain of paddy straw Mushroom (*Volvariella volvacea*). J. Plant Biochem. Biotechnol. 17(1): 57-63.

Allen S, Grimshay HM, Porkinson JA, Quarmby C (1974). Chemical analysis of ecological materials. Blackwell Scientific publication, Oxford, London, p. 565.

Angeli JPF, Riberiro LR, Bellini LR, Mantovani MS (2009). B-Glucan extracted from the medicinal mushroom (*Agaricus blazei*) prevents the genotoxic effects of benzo (a) pyrene in the human hepatoma cell line Hep G2. Arch. Toxicol. 83: 81-86.

Bashandy AS, El-Hifnawi HN (2002): Effect of physiological age and dose rate on radiation resistance of some radioresistant bacterial species. Az. J. Microbiol. 1: 61-67.

Bohn JA, BeMiller JNI (1995). β-D-glucan as biological response modifiers: A review of structure-functional activity relationship. Carbohydr. Polymers, 28: 3-14.

Borchers AT, Keen, GL, Gershwin ME (2004). Mini review-mushrooms Tumors and immunity: an update. Exp. Biol. Med. 229(5): 393-406.

Cammarano P, Felsani A, Gentile, M (1972). Formation of active hybrid 80-S particles from subunits of pen seedlings and mammalian liver ribosomes. Biochem. Biophys. Acta. 281: 625-642.

Clark JM, Switzer RL (1977). In Experimental biochemistry 2nd edn, free man company – San Francisco. p. 180.

Craig B, Alt FW (2004). The cell response to general and programmed DNA double strand breaks, DNA Repair, 3: 781-796.

Czop JK, Kay J (1991). Isolation and characterization of β-glucan receptors on human mononuclear phagocytes. J. Exp. Med. 173: 1511-20.

Dawoud MEA (1986). Role of some heavy metals in fungal growth. Ph. D. thesis Cairo Univ. Faculty of Science. Botany Department, pp. 27-32.

Dawoud MEA, Eweis M (2006). Phytochemical control of mushrooms pathogenic bacteria. J. Food, Agric. Environ. (Sci. Technol.). 4 (1): 321-324.

Dewey DL (1960). Effect of oxygen and nitric oxide on the radiosentivity of human cells in tissues culture. Nature, (London). 186: 780.

Dufosse L, De La Broisse D, Guerard F (1997). Fish protein hydrolysates as nitrogen source for microbial growth and metabolite production. Recent Res. Dev. Microbiol. 5: 365-381.

El-Mongy TM, El-Fouly MZ, Abo-Shady MR, Abd-El Aziz AB (2001). Effect of gamma irradiation on single cell protein (SCP) production and it's keeping quality, Az. J. Microbiol. 51: 242-251.

El-Hifnawi HN (2002). Influence of low doses of gamma radiation on the growth and sensitivity of *Staphyloccus aureus* (ATCC 25923) to antibiotics. Az. J. Microbiol. 55: 207-214.

El-Sayed EA, El-Ashmawy AM, Galal AM, Hassan IA (2001). combined effect of gamma irradiation and thiabendozole treatment on scoparone accumulation and rot caused by *Penicillium italicum* and *Aspergillus flavus* in grape fruit. Az. J. Microbiol. 53: 11-22.

Facq Z, Alexander P (1961). Foundmental of radiobiology. Pergamon press, Elmsford, NY.

Gregori A, Savagelj M, Pohleven J (2007). Cultivation technique and medicinal properties of pleurotus spp. Food Technol. Biotechnol. 45: 238-249.

Hugo WB (1995). A brief history of heat, chemical and radiation preservation and disinfection. Int. Biodeterior. Biodegr. 36(3): 197-217.

Hussein AM, Swelin MA, Ayatt NM, Yassin MH, El-Metwally MM (2002). Potentiality of some fungi for producing cellular proteins from rice hull black liquor hemicellulose. Az. J. Microbial. 57: 137-146.

Kadioglu A, Algur OF (1992). Tests of media with vinasse for *Chlamydomonas reinhardü* for possible reduction in vinasse pollution. Bioresour. Technol. 42: 1-5.

Kim DH, Yang BK, Jeong SC, Park JB, Cho SP, Das S (2001). Production of a hypoglycemic, extracellular polysaccharides from the submerged culture of the mushroom *Phellinus linteus*. Biotechnol. Lett. 23: 513-517.

Kim MS, Park KM, Chang IS, Sim YC (2000). β -(1, 6) Branched β -(1, 3)- glucan in skin care. Cosmet. Toil. 115: 79-86.

Kosaric N, Miyala N (1981). Growth of morel mushroom mycelium in cheese whey. J. Dairy Res. 48: 149-162.

Lavi L, Levinson D, Peri L, Tekoah Y, Harder Y, Schwartz B (2010). chemical characterization, antiproliferative and antiadhesive properties of polysaccharides extracted from *Pleurotus pulmonarius* mycelium and fruiting bodies. Appl. Microbiol. Biotechnol. 85(6): 1977-1990.

Lowry OH, Rosenbrough J, Fan AC, Randal RJ (1951). Protein measurements with folin phenol reagent. J. Biol. Chem. 193: 265-275.

Mantovani MS, Bellini MF, Angeli JPF, Oliveira RJ, Silva AF, Ribeiro LR (2008). β Glucans in promoting health: prevention against mutation and cancer. Mutat. Res. Rev. Mutat. Res. 658(3): 154-161.

Manzi P, Marconi S, Aguzzi A, Pizzoferrato L (2004). Commercial mushrooms nutritional quality and effect of cooking. Food Chem. 84: 201-206.

Meng C, Shi S, Lin H, Chen J, Guo Y (2007). UV induced mutation of *Acidianus brierleyi* growing in a continuous stirred tank reactor generated a strain with improved bioleaching capabilities. Enzyme Microbial Technol. 40: 1136-1140.

Paulik S, Mogzisova J, Durove A, Benisek Z, Husk M (1996). The immunomodulatory effect of the soluble fungal glucan (*Pleurotus ostreatus)* on delayed hypersensitivity and phagocytic ability of blood leucocytes in mice. J. Vet. Med. 43(3): 129-135.

Prasad KN (1995). Hand book of radiation biology; CRC Press, Boca Raton, New York, London, Tokyo. Second edition, pp. 50-125.

Ragunathan R, Gurusamy R, Palaniswamy M, Swaminathan K (1996). Cultivation of *Pleurotus* spp. On various agro-residues. Food Chem. 55: 130-144.

Razak AA (1980). Chemtaxonomy and biosynthetic pathway of several non-protein amino acids in plants. Ph. D. London University.

Richel P, Kovacocyova K, Sorocinova J, Dobolyova S, Seidel H, Huska M, Link R (2011). Feeding of energetic food admixture containing β glucan and it's effect on selected blood serum indices in cows and their calves in peripartal period. Veterinarska, 42(1): 58-64.

Said H, Naguib MI (1964). Sucrose estimation as a mean of estimation of the draw back taxon exported Halawa Tehinia. Bull. Fac. Cairo Univ. 39: 207-216.

Shen J, Re H, MiyaĴi MC, Sugay ST, Kuwano Y, Liai T, Hatakeyama K, Abo T (2007). Potentiation of intestinal immunity by micellary mushroom. Biomed. Res. 28(2): 71-77.

Sher H, Al-Yemeni M, Khan K (2011). Cultivation of the oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm.) in two different agroecological zones of Pakistan. Afr. J. Biotechnol. 10(2): 183-188.

Smiderlie RF, Olsen LM, Carbonero ER, Bagio CH, Freitas CS, Marcon R (2008). Antiinflammatory and analgesic properties in a rodent model of a (1 → 3), (1 → 6)-linked β-glucan isolated from *Pleurotus pulmonarius*. Eur. J. Pharmacol. 597: 86-91.

Snedcor GW, Cochran WG (1980). Statistical Methods, 7th ed. Iowa state Univ. press Iowa, USA.

Synytsya A, Mickova K, Synytsya A, Jablonsky I, Spevacek J, Erban V, Kovarikova E, copikova J (2009). Glucan from fruit bodies of cultivated mushroom *Pleurotus ostreatus* and *Pleurotus eryngo*: structure and perbiotic activity. Carbohydr. Polymers, 76: 548-556.

Viladomat FJM, Elabres J, Bastida RM, Cusido C, Codina (1986). Ontogenic variations in the alkaloids of *Narcissus assoanus.* Physiol Plant, 68: 657-661.

Walter A, Duncan DB (1969). Multiple range and multiple test. Biometrics, 11: 1-24.

Ward G, Johnson FS (1962). Salt resistance. Canad. Dep. Agric. 10(64): 1-59.

Wasser SP (2002). Medicinal mushrooms as a source of anti-tumor and immunomodulating polysaccharides. Appl. Microbiol. Biotechnol. 60(3): 258-274.

Yanaki T, Ito W, Kojima T (1981). Ultrasonic degradation of schizophyllan, an antitumor polysaccharide produced by *Schizophyllum commune*. Fries Carbohydr. Res. 89: 121-35.

Younis ME, Hassaneen MNA, Nemet-Alla MM (1987). Plant growth metabolism and adaptation in relation to stress conditions. IV. Effect of salinity on certain factors associated with the germination of three different seeds high in fat. Ann. Bot. 60: 337-344.