Australian Journal of Basic and Applied Sciences, 4(11): 5717-5726, 2010
ISSN 1991-8178

Preparation of Glucan from *Lentinula edodes* Edible Mushroom and Elucidation of its Medicinal Value

[1]George E. Rasmy, [2]William A. Botros, [2]SanaaS. Kabeil and [2]Ayman. S. Daba

[1]Biochemistry Dept., National research centre (NRC), Cairo, Egypt.
[2]Mubarak City for scientific Research and Technology Applications, GEBRI, Alexandria, Egypt.

Abstract: One of the most popular mushrooms in the World is Shiitake mushroom (*lentinula edodes)*. In the present study *(L. edodes)* mycelia were grown in submerged culture and the polysaccharides were extracted from culture broth. The structure of polysaccharides was elucidated using NMR spectra, which indicated that the polysaccharide is highly branched glucan containing mainly 1, 3 and 1, 6 linkages. The results showed that the polysaccharides posses anticancer activity against human esophageal cancer cell line. The potential of the internal transcribed spacer (ITS) region as a tool for studying molecular systematics and population genetics is significant. The results also showed that the polysaccharides enhance the immune-responses of human body thereby increasing resistance to cancer disease. Mycelia formed by growing pure cultures in submerged conditions are of constant composition, and submerged culture is the best technique for obtaining consistent and safe mushroom product.

Key words: Mushroom, polysaccharides, immune-responses, submerged culture, cancer.

INTRODUCTION

Consumption of edible mushrooms has been suggested to improve health. There are thousands of different mushroom species and about 700 species have been reported to have significant pharmacological properties (Chang, 1996). Medicinal mushrooms have a long history of use in traditional Oriental therapies. Hot-water-soluble fractions of medicinal mushrooms have been used as medicine in the Far East (Wasser, 2002).

In the last three decades, numerous polysaccharides and polysaccharide-protein complexes have been isolated from mushrooms and used as a source of therapeutic agents. The most promising biopharmacological activities of these biopolymers are their immunomodulation and anti-cancer effects.

They are mainly present as glucans with different types of glycosidic linkages such as (1-->3), (1-->6)-beta-glucans and (1-->3)-alpha-glucans, and as true heteroglycans (figure 1), while others mostly bind to protein residues as polysaccharide-protein complexes (Chihara *et al.,* 1987 and Zheng *et al.,* 2005). The β-glucans have been shown to inhibit tumor growth in vitro and in vivo.

The β-glucans lentinan from L. edodes, schizophyllan (sonifilan) from Schizophyllum commune, grifolan from Grifola frondosa, and extracts from Sclerotinia sclerotiorum all have anti-tumor activity. Intratumor injection of an acid-treated fraction of Agaricus blazei inhibited tumor growth of that tumor as well as other tumors at remote sites (Fujimiya *et al*., 1998). An extract from the Phellinus rimosus mushroom extended the life span of mice by 96% following injection of tumor cells in an experimental Dalton's lymphoma ascites model (Ajith and Janardhanan, 2003).

L. edodes is the first medicinal macrofungus to enter the realm of modern biotechnology. It is the second most popular edible mushroom in the global market which is attributed not only to its nutritional value but also to possible potential for therapeutic applications. *L. edodes* is used medicinally for diseases involving depressed immune function (including AIDS), cancer, environmental allergies, fungal infection, frequent flu and colds, bronchial inflammation, heart disease, hyperlipidemia (including high blood cholesterol), hypertension, infectious disease, diabetes, hepatitis and regulating urinary inconsistencies. It is the source of several well-studied preparations with proven pharmacological properties, especially the polysaccharide lentinan, eritadenine, shiitake mushroom mycelium, and culture media extracts (LEM, LAP and KS-2). Antibiotic, anti-carcinogenic and antiviral compounds have been isolated intracellularly (fruiting body and mycelia) and extracellularly (culture media). Bisen *et al*., 2010.

Corresponding Author: Dr. George E. Rasmy, Biochemistry Dept., National research centre (NRC), Cairo, Egypt.
E-mail: gefahmy@gmail.com

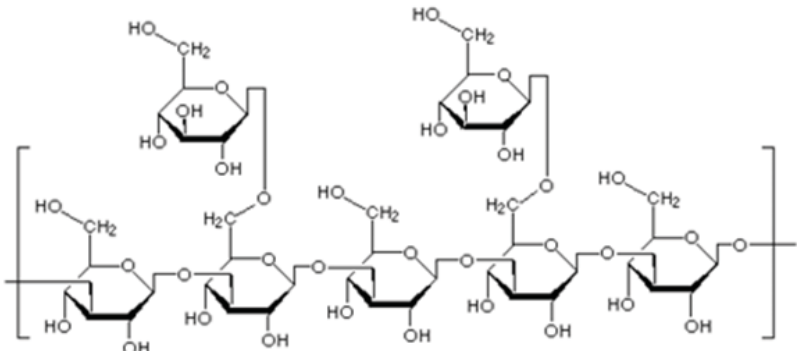

Fig. 1: Chemical structure of mushroom polysaccharides.

During the last 15 years the internal transcribed spacer (ITS) of nuclear DNA has been used as a target for analyzing fungal diversity in environmental samples, and has recently been selected as the standard marker for fungal DNA barcoding. Bellemain *et al*., (2010). The potential of ITS region as a tool for studying molecular systematics and population genetics is significant.

Hearst *et al*., 2009, previously suggested that it is required to isolate and identify active compound(s) in L. edodes. They stated that once these have been identified, suitable pharmaceutical delivery systems should be explored to allow concentrated extracts to be prepared and delivered optimally, rather than crude ingestion of raw material, which could promote further bacterial resistance.

The aims of the present study were to 1- growing *L. edodes* mycelia in submerged culture and extracting the polysaccharides from culture broth. 2- to elucidate the structure of polysaccharides using NMR spectra, and 3- to generate the phylogenetic tree of this isolate compared to other isolates. And finally 4- to posses the biological activity of the isolated polysaccharides both as antimicrobial and as anticancer (against human esophageal cancer cell line)

MATERIAL AND METHODS

Microorganism and Media

A culture of *L. edodes* was isolated from the forest in La Crosse USA. The culture was maintained on a yeast complete medium and subculture every 1 month, and the slants were incubated at 27ºC for 7 days and then stored at 4ºC. The seed cultures were grown in 250ml flasks containing 100 ml of medium containing (g/l) : glucose 10, yeast extract 1, peptone 2, KH2 PO4 0.5 , Mg SO4 0.5 at 27ºC on a rotary incubator at 150 rpm for 7 days.

Fermentation:

L. edodes was initially grown on yeast complete medium in Petri dish, and then transferred to the seed culture medium by punching out 2.5mm of the agar plate culture with a sterilized cutter. After 7 days of growing in shake flasks .The fermentation medium was inoculated by 3 % of the seed culture. A 15 liters Stirred tank fermentor (BIOFLO 3000 New Brunswick, USA) was used in the experiment, the cultivation in the fermentor for 10 days. The temperature was adjusted for 27ºC and the rpm 150. Fermentation experiment was performed in duplicate.

Extraction of the Polysaccharide:

The fermentation broth was filtered and then centrifuged at 5000 X g for 15 minutes, and the resulting supernatant was concentrated using rotary evaporator .The concentrated supernatant was mixed with two volume of absolute ethanol, stirred vigorously and left overnight at 4ºC. The purified polysaccharide is then pooled and lyophilized.

Estimation of Mycelial Growth and Polysaccharide Production:

Samples collected from the fermentor at various intervals were centrifuged at 5000 X g for 15 minutes. Polysaccharide was separated from supernatant and lyophilized until constant weight was confirmed. The dry

weight of mycelia was measured after repeated washing of the mycelial pellets and drying overnight at 70ºC to a constant weight.

NMR:

The ^{13}C NMR spectra were recorded on an INOVA-600 spectrometer (Varian, Palo Alto, CA, USA) at ambient temperature.

MTT Assay:

Human esophageal cancer cell line was kindly obtained from university of Cape Town.

The amount of yellow MTT (3- (4, 5-Dimethylthiazol- diphenyltetrazolium bromide) reduced to purple formazan is measured spectrophotometrically by a spectrometer. This reduction takes place only when mitochondrial reductase enzymes are active, and thus conversion is directly related to the number of viable cells. The production of purple formazan in cells treated with an agent is measured relative to the production in control cells, and a dose-response curve can be generated (Mosmann, 1983).

Antimicrobial Activity Assay:

Muller-Hinton agar medium was used as an assay medium. The agar medium at 45ºC was mixed with 0.1ml bacterial suspension. The mixture was powered into 9cm petri dish and allow to ceritified. Sterile paper diskes were placed on the dried surface of the medium. Each disk received 20ml of the culture filtrate. Perti dishes were incubated at 37ºC for 18hs. The inhibation zone was measured mm. diameter (Amade *et al.,* 1994).

DNA Extraction:

DNA extraction was done using C-TAB Lysis buffer and 70% ethanol precipitation. DNA clean up via Geneclean III kit by Q-biogene Ten microlitres of each of the extracted DNA solutions were checked by an electrophoresis on a one percent agarose gel.

PCR Analysis:

Total DNA was subject with primers specific to the ITS-domain (5'-CTTGGTCATTTAGAGGAAGTAA-3'), (5'-CCTCCGCTTATTGATATGC-3') (White *et al.,* 1990). DNA amplifications were carried out in a thermocycler Eppendourf PCR system with denaturing step at 95ºC for 5min and the step cycle program set for 35 cycles (with a cycle consisting of denaturing 94ºC for 30s, annealing at 45ºC for 30s and extension step at 72ºC for 30s), followed by a final extension step at 72ºC for 10 min.

Cloning and Sequencing of PCR Fragments:

Expected PCR-amplified fragments were excised from agarose gel and purified using Qiagen Gel Extraction kit (Qiagen, Germany) and then cloned with TOPO TA cloning kit (invetrogen, USA) in the competent E. coli strain TOPO 10. Plasmid DNA was isolated using Qia Spin mini-prep kit (Qiagen, Germany). Plasmid DNA was sequenced in both directions using BigDye Sequencing kit and Applied Biosystems 3730xl automated DNA sequencing Instrument at the University of Wisconsin, USA. Biotechnology Center.

Alignment and Phylogeny:

Pairwise and multiple DNA sequence alignment were carried out using CULSTALW (http://align.genome.jp). The phylogenetic tree was generating using MUSCALE 3.7 (http://www.phylogeny.fr/) according to Dereeper *et al.,* 2008). To obtain the phylogeny tree, 10 different strains were used, including ours, as illustrated in table (1).

RESULTS AND DISCUSSION

Shiitake (L. edodes (Berkeley) Pegler) is one of the most consumed mushrooms, for both therapeutic purposes and as food, therefore, the study of its biological properties is of great interest for producers and consumers. L. edodes mycelia have an excellent nutritional value. Their raw mycelia were found to include 88–92% water, protein, lipids, carbohydrates as well as vitamins and minerals. On a dry weight basis, they have a relatively high nutritional value when compared to commonly consumed vegetables. Dried shiitake mushrooms are rich in carbohydrates and protein. They contain 58–60% carbohydrates, 20–23% protein

Table 1: Strains of L. edodes or P. ostreatus used to obtain Alignment and phylogeny.

geneBank #	*strain*	Reference
AB366150.1	*L. edodes* strain: G408PP-4 , 18S rRNA	Miyazaki *et al*., 2007
EU424320.2	*L. edodes* strain ACCC51462, 18S rRNA gene, partial sequence	Gao *et al*., 2008
EU520255.1	*P. ostreatus* isolate NW438 18S rRNA gene, partial sequence	Yu, 2008
FJ481023.1	*L. edodes* strain xsd08112, 18S rRNA gene, partial sequence	Jiang *et al*., 2008
FJ582641.1	*L. edodes* strain xsd08092 18S rRNA gene, partial sequence	Jiang *et al*., 2008
FJ810189.1	*L. edodes* strain dd08110, 18S rRNA gene, partial sequence	Jiang *et al*., 2009
GU001952	*L. edodes* strain 135, 18S rRNA gene, partial sequence	Cao & Bao, 2009
HQ186261	*L. edodes* 18S rRNA gene, ITS1, 5.8S rRNA gene ITS2 and 28S rRNA gene	Le *et al*., 2010
U33091.1	*L. edodes* isolate TMI-818	Hibbett *et al*., 1995

(digestibility of 80–87%), 9–10% fiber, 3–4% lipids, and 4–5% ash (Data not shown). Shiitake is one of the best-known and best-characterized mushrooms used in medicine. It is the source of several well-studied preparations with proven pharmacological properties, especially the polysaccharide. Using methods of fractionation and purification of polysaccharide reported by Chihara (1969 and 1992) exo-polysaccharides were isolated from shiitake, Chihara was one of the first to report on the anti-tumor properties of the mushroom, stating that lentinan a polysaccharide isolated from the fruit bodies ''was found to almost completely regress the solid type tumors of Sarcoma 180 and several kinds of tumors including methylchloranthrene induced fibrosarcoma in synergic host–tumor system Wasser (2002).

The potential of the ITS region as a tool for studying molecular systematics and population genetics is significant (Boyer *et al.,* 2001). We compared our ITS r-RNA *L. edodes* sequence with another sequences on GenBank to detect the similarity of this region with other *L. edodes* isolates (figures 2, 3) (Hibbett *et al.,* 1995; Miyazaki *et al.,* 2007, Gao *et al*., 2008, Yu, 2008, Jiang *et al*., 2008 (a), Jiang *et al*., 2008 (b), Jiang *et al*., 2009, Cao & Bao, 2009, and Le *et al*., 2010).

The H NMR Spectra (Figure4) of the exopolysaccharides exhibited signals at different resonance which represent the anomeric proton and protons of the different hydroxyl groups. The C13 NMR Spectra showed the presence of 6 carbon atoms the proton magnetic resonance is the most accurate spectroscopic method used to determine the structure of new compounds. The obtained NMR spectra could be compared with those reported by Gorin (1981) for lentinan polysaccharides, a potent antitumor agent extracted from shiitake.

Mushroom polysaccharides were tested for antibacterial activity in vitro against gram positive and gram negative bacteria. The minimum inhibitory concentration reported in Table (2) shows that mushroom polysaccharide has a potent antibacterial effect against different kind of bacteria. Only a few studies have explored shiitake's antibacterial components, and these have concentrated on their potential in terms of bacteria of oral origin. Anti-bacterial activity is an exciting result, with increasing bacterial resistance to antibiotics, improving host immunity may be the way forward in fighting bacterial infection. But, these results should be viewed with caution because the published papers in this area are confined to only one or two journals, and although these have reliable IF scores, this fact does not lend support to them being credible results. There is a lack of significant studies in this area; and none that were from researching the anti-caries aspect of anti-bacterial activity.

A postulated mechanism of lentinan's anti-bacterial activity was by the induction of increased levels of complement C3 and C3b formation (Shouji *et al.,* 1999). Although, modulation of the non-specific immune system has also been displayed in numerous studies, and may be the potentiator of lentinan's anti-bacterial activity.

Cytotoxic activity of isolated polysaccharides was examined using esophageal cancer cell line in vitro. Results showed that polysaccharides inhibited tumour cell growth .This result are with the conclusion of other investigators (Hibasami *et al*., 2003 and Zhang *et al*., 2007) who stated that these polysaccharides could arrest the cell cycle and generate apoptosis. Also Miyaji *et al*., 2006, evaluated the aqueous extracts of the shiitake mushroom (*L. edodes* (Berkeley) Pegler) in HEp-2 cells in vitro. Jwanny *et al*., 2009, also found a strong cytotoxic effect of polysaccharides isolated from *pleurotus ostreatus* and *Trigonella foenumgraecum* on different cell lines i.e. HCT-116 (colon), HepG2 (liver) and U251 (brain)

Extracts of multiple varieties of mushrooms have been shown to be protective in experimental cancer models; presumably because in part they boost anti-tumor immunity. These polysaccharides and polysaccharide-protein complexes are suggested to enhance cell-mediated immune responses in vivo and in vitro and act as biological response modifiers (Borchers *et al.,* 1999). Potentiation of the host defense system may result in the activation of many kinds of immune cells that are vitally important for the maintenance of homeostasis. Polysaccharides or polysaccharide-protein complexes are considered as multi-cytokine inducers that are able

Gblocks 0.91b Results, Processed file: input.fasta
Number of sequences: 10, New number of positions: 582

```
                  10        20        30        40        50        60
                  +---------+---------+---------+---------+---------+
U33091.1          CCTCCGATTTCTATTCATCCACCTGTGCACTTTTTGTAGGAGTTCTTTCATCGGGTTTTT
HQ186261          CCTCCGATTTCTATTCATCCACCTGTGCACTTTTTGTAGGAGTTCTTTCATCGGGTTTTT
EU520255.1        CCTCCGATTTCTATTCATCCACCTGTGCACTTTTTGTAGGAGTTCTTTCATCGGGTTTTT
GU001952          CCTCCGATTTCTATTCATCCACCTGTGCACTTTTTGTAGGAGTTCTTTCATCGGGTTTTT
EU424320.2        CCTCCGATTTCTATTCATCCACCTGTGCACTTTTTGTAGGAGTTCTTTCATCGGGTTTTT
ITS_L._Edodes     CCTCCGATTTCTATTCATCCACCTGTGCACTTTTTGTAGGAGTTCTTTCATCGGGTTTTT
FJ810189.1        CCTCCGATTTCTATTCATCCACCTGTGCACTTTTTGTAGGAGTTCTTTCATCGGGTTTTT
AB366150.1        CCTCCGATTTCTATTCATCCACCTGTGCACTTTTTGTAGGAGTTCTTTCATCGGGTTTTT
FJ582641.1        CCTCCGATTTCTATTCATCCACCTGTGCACTTTTTGTAGGAGTTCTTTCATCGGGTTTTT
FJ481023.1        CCTCCGATTTCTATTCATCCACCTGTGCACTTTTTGTAGGAGTTCTTTCATCGGGTTTTT
                  ############################################################

                          70        80        90       100       110       120
                          +---------+---------+---------+---------+---------+
U33091.1          GAA-GGTGCTCATTATGAGTTAC-TGAAAAGACTAGTTGAC-AGGCTTCTATGTTTCTTA
HQ186261          GAAGGGTGCTCATTATGAGTTACTTGAAAAGACTAGTTGACAAGGCTTCTATG-TTCTTA
EU520255.1        GAA-GGTGCTCATTATGAGTTACTTGAAAAGACTAGTTGACAAGGCTTCTATG-TTCTTA
GU001952          GAA-GGTGCTCATTATGAGTTACTTGAAAAGACTAGTTGACAAGGCTTCTATG-TTCTTA
EU424320.2        GAA-GGTGCTCATTATGAGTTACTTGAAAAGACTAGTTGACAAGGCTTCTATG-TTCTTA
ITS_L._Edodes     GAA-GGTGCTCATTATGAGTTACTTGAAAAGACTAGTTGACAAGGCTTCTATG-TTCTTA
FJ810189.1        GAA-GGTGCTCATTATGAGTTACTTGAAAAGACTAGTTGACAAGGCTTCTATG-TTCTTA
AB366150.1        GAA-GGTGCTCATTATGAGTTACTTGAAAAGACTAGTTGACAAGGCTTCTATG-TTCTTA
FJ582641.1        GAA-GGTGCTCATTATGAGTTACTTGAAAAGACTAGTTGACAAGGCTTCTATG-TTCTTA
FJ481023.1        GAA-GGTGCTCATTATGAGTTACTTGAAAAGACTAGTTGACAAGGCTTCTATG-TTCTTA
                  ### ################## ################ ########### ######

                        130       140       150       160       170       180
                        +---------+---------+---------+---------+---------+
U33091.1          TAAACCATTGAAGTATG-TATAGAATGATCTTGTTATTGGGACTTTATTGA-CCTTTAAA
HQ186261          TAAACCATTGAAGTATGTTATAGAATGATCTTGTTATTGGGACTTTATTGACCCTTTAAA
EU520255.1        TAAACCATTGAAGTATGTTATAGAATGATCTTGTTATTGGGACTTTATTGACCCTTTAAA
GU001952          TAAACCATTGAAGTATGTTATAGAATGATCTTGTTATTGGGACTTTATTGACCCTTTAAA
EU424320.2        TAAACCATTGAAGTATGTTATAGAATGATCTTGTTATTGGGACTTTATTGACCCTTTAAA
ITS_L._Edodes     TAAACCATTGAAGTATGTTATAGAATGATCTTGTTATTGGGACTTTATTGACCCTTTAAA
FJ810189.1        TAAACCATTGAAGTATGTTATAGAATGATCTTGTTATTGGGACTTTATTGACCCTTTAAA
AB366150.1        TAAACCATTGAAGTATGTTATAGAATGATCTTGTTATTGGGACTTTATTGACCCTTTAAA
FJ582641.1        TAAACCATTGAAGTATGTTATAGAATGATCTTGTTATTGGGACTTTATTGACCCTTTAAA
FJ481023.1        TAAACCATTGAAGTATGTTATAGAATGATCTTGTTATTGGGACTTTATTGACCCTTTAAA
                  ################ ################################## ########

                              190       200       210       220       230       240
                              +---------+---------+---------+---------+---------+
U33091.1          CTTAATACAACTTTCAGCANCGGATCTCTTGGCTCTCCCATCGATGAAGAACGCAGCGAA
HQ186261          CTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCCCATCGATGAAGAACGCAGCGAA
EU520255.1        CTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCCCATCGATGAAGAACGCAGCGAA
GU001952          CTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCCCATCGATGAAGAACGCAGCGAA
EU424320.2        CTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCCCATCGATGAAGAACGCAGCGAA
ITS_L._Edodes     CTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCCCATCGATGAAGAACGCAGCGAA
FJ810189.1        CTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCCCATCGATGAAGAACGCAGCGAA
AB366150.1        CTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCCCATCGATGAAGAACGCAGCGAA
FJ582641.1        CTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCCCATCGATGAAGAACGCAGCGAA
FJ481023.1        CTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCCCATCGATGAAGAACGCAGCGAA
                  ############################################################

                              250       260       270       280       290       300
                              +---------+---------+---------+---------+---------+
U33091.1          ATGCGATANGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTT
HQ186261          ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTT
EU520255.1        ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTT
GU001952          ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTT
EU424320.2        ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTT
ITS_L._Edodes     ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTT
FJ810189.1        ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTT
AB366150.1        ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTT
FJ582641.1        ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTT
FJ481023.1        ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTT
                  ############################################################

                              310       320       330       340       350       360
                              +---------+---------+---------+---------+---------+
U33091.1          GCGCCCTCTGGTATNCCGGA-GGNAT-CCTGTTTGAGTGTCATTAAATTCTCAACTTTAT
HQ186261          GCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACTTTAT
EU520255.1        GCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACTTTAT
GU001952          GCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACTTTAT
EU424320.2        GCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACTTTAT
ITS_L._Edodes     GCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACTTTAT
FJ810189.1        GCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACTTTAT
AB366150.1        GCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACTTTAT
FJ582641.1        GCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACTTTAT
FJ481023.1        GCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACTTTAT
                  ##################       ################################
```

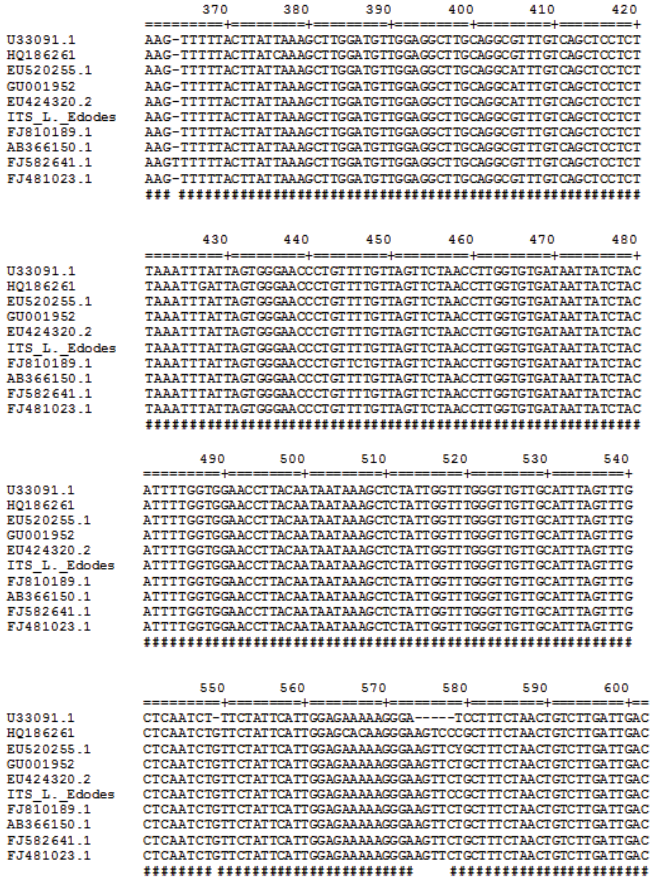

Fig. 2: Alignment of ITS (internal transcribed spacer) rRNA region from a single isolate of L. edodes with different species

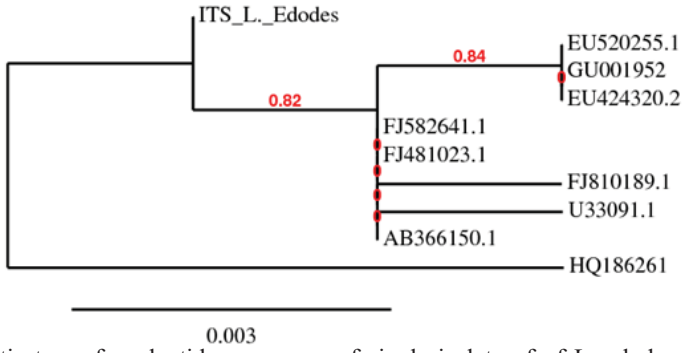

Fig. 3: Phylogenetic tree of nucleotide sequences of single isolate of of L. edodes with different species

to induce gene expression of various immunomodulatory cytokines and cytokine receptors (Okamoto *et al.,* 2004).

Some interesting studies focus on investigation of the relationship between their structure and antitumor activity, elucidation of their antitumor mechanism at the molecular level, and improvement of their various biological activities by chemical modifications. Israilides *et al* (2008) demonstrate cytotoxic and cell growth inhibitory (cytostatic) effect of aqueous extracts of the shiitake mushroom on MCF-7 human breast adenocarcinoma cell line. Such effect was demonstrated with fruit body and mycelial extracts, the difference

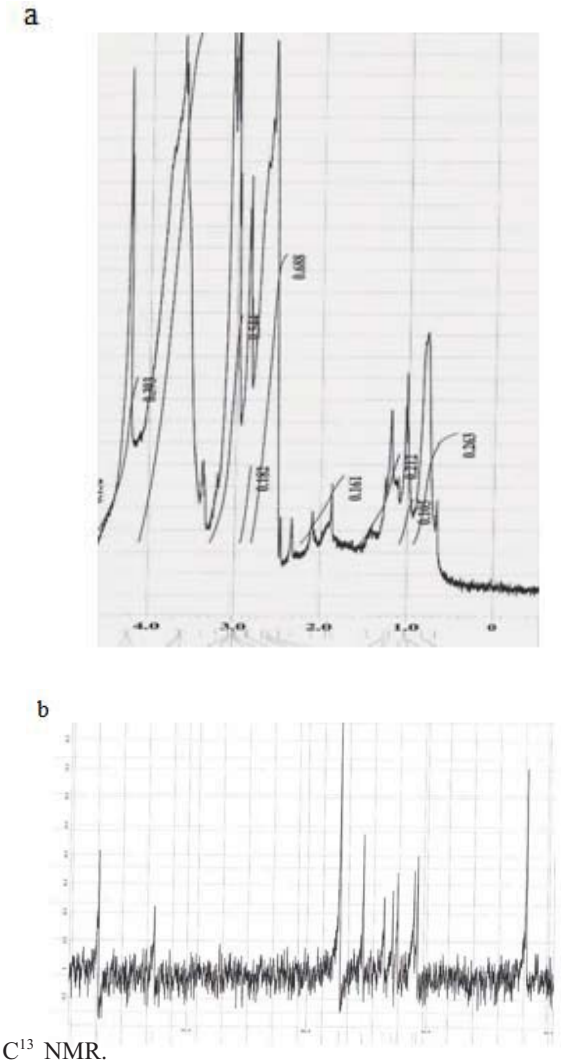

Fig. (4 a,b): Proton and C^{13} NMR.

Table 2: Antimicrobial activity of Shiitake polysaccharides

Test bacterium	MIC (mg) for sample
Gram Positive	
Bacillus coagulans ATCC 7050	-
Bacillus megraterium NCIB 2602	150
Enterococcus faecium vanA	-
Enterococcus phoeniculicola JLB-1T	150
Micrococcus sp.	100
Mycobacterium aurum A+	300
Mycobacterium tuberculosis H37Rv ATCC 27294	-
Staphylococcus aureus	-
Streptococcus sp.	150
Gram negatives:	
Acinetobacter calcoaceticus C91	150
Citrobacter braaki 90	-
Enterobacter cloacae 67	150
Escherichia coli ATCC 25922	-
Escherichia coli ATCC 35218	-
Klebsiella oxytoca K52	150
Klebsiella peneumoniae K4	80
Proteus mirabilis 87	-
Pseudomonosa ATCC 27853nas aerugi	-

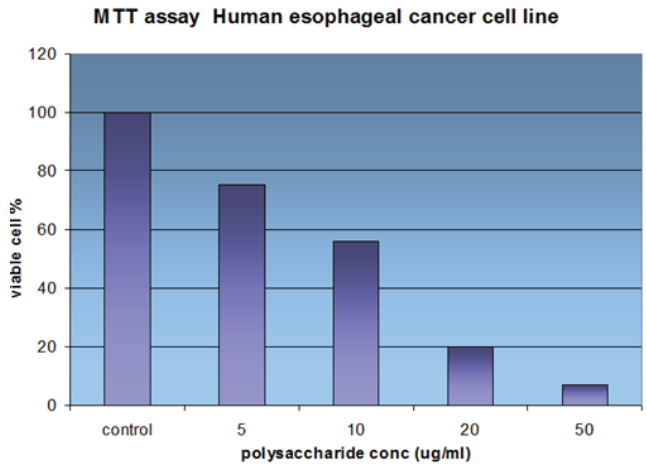

Fig. 5: Cytotoxic activity of Shiitake polysaccharides assessed by the Mossman assay against human esophageal cancer cell line. The bars report the percentage of viable cells after 24 hours of their exposure to different conc. of polysaccharides compared control.

being that there was no significant suppression on normal cells with the latter. Furthermore mycelial extracts did not induce any cytostatic effect in both cancer and normal cell lines based on a DNA synthesis assay. The significant suppression of the proliferation of cancer cells was reflected by the comparatively low IC (50) values and the simultaneous higher respective values on normal fibroblast cells.

There is a lot of evidence to support the anti-tumour assertions made of lentinan. A number of valuable studies have been conducted on the consequence of lentinan administration, and its acceptance into clinical medicine in Japan should perhaps highlight its efficacy. The researchers in Japan have covered much ground in the area of neutraceuticals from mushrooms and have unearthed the potential of *L. edodes*. At the moment there is not enough credible information to warrant the marketing of lentinan in the world, further investigations, look to be promising, while isolated purified shiitake constituents have received appropriate scrutiny investigations into the anti-tumour potentials of shiitake consumption are limited. There is a lack of epidemiological data as to the prophylactic effects of mushroom intake on the development of spontaneous tumours and the evidence that suggests shiitake consumption elicits anti-tumour effects are only found in murine systems. Therefore, the claim that shiitake consumption has anti-tumour effects is not fully substantiated.

Conclusions:

This study shows the potential the efficacy of the given method to isolate a highly branched glucan containing mainly 1, 3 and 1, 6 linkages. Biological examinations revealed its antimicrobial, cytotoxic activity against esophageal cancer cell line. Further research is needed to establish content and bioactivity of the many compounds present and the effect of preparation and consumption differences on their medicinal activity.

REFERENCES

Ajith, T., K.K. Janardhanan, 2003. Cytotoxic and antitumor activities of a polypore macrofungus, Phellinus rimosus (Berk) Pilat. J Ethnopharmacol, 84: 157-162.

Amade, P., M. Mallea and N. Bouaicha, 1994. Isolation, structural identification and biological activity of two metabolites produced by penicillium olsonii Bainier and Sartory. J. Antibiot., 47: 201-207.

Bellemain, E., T. Carlsen, C. Brochmann, E. Coissac, P. Taberlet, H. Kauserud, 2010. ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC Microbiol., 9(10): 189.

Bisen, P.S., R.K. Baghel, B.S. Sanodiya, G.S. Thakur, G.B. Prasad, 2010. Lentinus edodes: a macrofungus with pharmacological activities. Curr Med Chem., 17(22): 2419-30.

Borchers, A.T., J.S. Stern, R.M. Hackman, C.L. Keen and M.E. Gershwin, 1999. Mushrooms, tumors, and immunity. Proc Soc Exp Biol Med., 221(4): 281-93.

Boyer, S.L., V.R. Flechtner and J.R. Johansen, 2001. Is the 16S–23S rRNA Internal Transcribed Spacer Region a Good Tool for Use in Molecular Systematics and Population Genetics? A Case Study in Cyanobacteria. Mol. Biol. Evol., 18(6): 1057-1069.

Cao, M. and D.P. Bao, Submitted 24-SEP-2009. Lentinula edodes strain 135 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence. (http://www.ncbi.nlm.nih.gov/nuccore/261347180). GenBank: GU001952.1

Chang, R., 1996. Functional properties of edible mushrooms. Nutr Rev., 54: S91-93.

Chihara, G., 1969. The antitumor polysaccharide lentinan: an overview. In T. Aoki, *et al*. (Eds.), Manipulation of host defense mechanism. Nature, 222: 687-694.

Chihara, G., 1992. Immunopharmacology of lentinan, a polysaccharide isolated from lentinus edodes: its application as a host defense potentiator. International Journal Oriental Medicine, 17: 57-77.

Chihara. G., J. Hamuro, Y. Maeda, *et al*., 1987. Antitumor and metastasis inhibitory activities of lentinan as an immunomodulator. Cancer Detect Prev., 1: 423-43.

Dereeper, A., V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J.F. Dufayard, S. Guindon, V. Lefort, M. Lescot, J.M. Claverie and O. Gascuel, 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist Nucleic Acids Research.; 36 (Web Server Issue):W465-9.

Fujimiya, Y., Y. Suzuki, K. Oshiman, H. Kobori, K. Moriguchi, H. Nakashima, Y. Matumoto, S. Takahara, T. Ebina, R. Katakura, 1998. Selective tumoricidal effect of soluble proteoglucan extracted from the basidiomycete, Agaricus blazei Murill, mediated via natural killer cell activation and apoptosis. Cancer Immunol Immunother, 46: 147-159.

Gao, S., J.X. Zhang and Y.B. Bian, Submitted 24-JAN-2008. Lentinula edodes strain ACCC51462 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence (http://www.ncbi.nlm.nih.gov/nuccore/257127897). GenBank: EU424320.2

Gorin, J., 1981. C13 NMR spectroscopy of polysaccharides In Advances in carbohydrate chemistry and biochemistry, Stuart, L and Derk, H (eds), New York, Academic Press, pp: 51.

Hearst, R., D. Nelson, G. McCollum, B.C. Millar, Y. Maeda, C.E. Goldsmith, P.J. Rooney, A Loughrey, J.R. Rao, J.E. Moore, 2009. An examination of antibacterial and antifungal properties of constituents of Shiitake (Lentinula edodes) and oyster (Pleurotus ostreatus) mushrooms. Complement Ther Clin Pract., 15(1): 5-7.

Hibasami, H., H. Moteki, K. Ishikawa, *et al*., 2003. Protodioscin isolated from fenugreek (Trigonella foenum graecum L.) induces cell death and morphological change indicative of apoptosis in leukemic cell line H-60, but not in gastric cancer cell line KATO III. Int J Mol Med., 11: 23-6.

Hibbett, D.S., M.J. Donoghue, A. Tsuneda and Y. Fukumasa-Nakai, 1995. Lentinula edodes isolate TMI-818 , 5.8S rRNA gene, complete sequence, 18S rRNA and 25S rRNA genes, partial sequence. Mycologia, 87(5): 618-638.

Israilides, C., D. Kletsas, D. Arapoglou, A. Philippoussis, H. Pratsinis, A. Ebringerová, V. Hríbalová and S.E. Harding, 2008. In vitro cytostatic and immunomodulatory properties of the medicinal mushroom Lentinula edodes. Phytomedicine, 15: 512-9.

Jiang, J., X. Cao, Y. Sun, X. Ju, T. Yin, Q. Miao, C. Li and C. Submitted 22-NOV-2008a. The Key Laboratory of Biotechnology for Medicinal Plants of Jiangsu, Lentinula edodes strain xsd08112 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence. (http://www.ncbi.nlm.nih.gov/nuccore/218664616) GenBank: FJ481023.1

Jiang, J., X. Ju, X. Cao, Y. Sun, T. Yin and Q. Miao, Submitted 19-DEC-2008b. Lentinula edodes strain xsd08092 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence. (http://www.ncbi.nlm.nih.gov/nuccore/221145757). GenBank: FJ582641.1

Jiang, J., S. Zhang, X. Cao, T. Yin, Q. Miao, C. Li, K. Mei and Y. Hu, submitted 09-MAR-2009. The Key Laboratory of Biotechnology for Medicinal Plants of Jiangsu. Lentinula edodes strain dd08110 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence (http://www.ncbi.nlm.nih.gov/nuccore/226346643). GenBank: FJ810189.1

Jwanny, E.W., S.A. Moharib and G.E. Rasmy, 2009. Effect of two polysaccharides on chemically-induced colorectal cancer in rats Etidal E. Advances in food science, 31(4): 202-209.

Le, P.L.H., T.L.T. Nguyen, A.T.H. Le and N.B. Truong, Submitted 26-AUG-2010. Lentinula edodes 18S ribosomal RNA gene, partial sequence; ITS 1, 5.8S ribosomal RNA gene, and ITS 2, complete sequence; and 28S ribosomal RNA gene, partial sequence. Available on pubmed website (http://www.ncbi.nlm.nih.gov/nuccore/308055645) GenBank: HQ186261.1

Miyazaki, K., Y. Tsuchiya and T. Okuda, Submitted 31-OCT-2007. Forest Microbiology Group. Lentinula edodes genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 25S rRNA, partial and complete sequence, strain: G408PP-4. Available on pubmed genebank website (http://www.ncbi.nlm.nih.gov/nuccore/187369517), GenBank: AB366150.1.

Mosmann, T., 1983. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. J. Immunol. Meth., 65: 55-63.

Okamoto, T., R. Kodoi, Y. Nonaka, I. Fukuda, T. Hashimoto, K. Kanazawa, M. Mizuno and H. Ashida, 2004. Lentinan from shiitake mushroom (Lentinus edodes) suppresses expression of cytochrome P450 1A subfamily in the mouse liver. Biofactors, 21: 407-9.

Shouji, N., K. Takada, K. Fukushima, M. Hirasawa and N. Tomotake, 1999. Three kinds of antibacterial substances from Lentinus edodes. International Journal of Antimicrobial Agents, 11: 151-157.

Wasser, S.P., 2002. Medicinal mushroom as a source of antitumour and immunomodulating polysaccharides. App.Microbiol. Biotechnology, 60: 258-274.

White, T.J., T. Bruns, S. Lee and J.W. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 In: PCR Protocols: A Guide to Methods and Applications, eds. Innis, M.A., D.H. Gelfand, J.J. Sninsky, and T.J. White. Academic Press, Inc., New York.

Yu, Z., Submitted 22-FEB-2008. Pleurotus ostreatus isolate NW438 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence. Northwest University of Agriculture and Forestry, College of Forestry. (http://www.ncbi.nlm.nih.gov/nuccore/189162246) GenBank: EU520255.1

Zhang, M., S.W. Cui, P.C.K. Cheung and Q. Wang, 2007. Wang Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. Trends in Food Science & Technology, 18: 4-19.

Zheng, R., S. Jie, D. Hanchuan and W. Moucheng, 2005. Characterization and immunomodulating activities of polysaccharide from Lentinus edodes. Int Immunopharmacol., 5(5): 811-20.